

Modulation of starch digestion for productive performance in dairy cows

by
Maria Ndakula Tautiko Shipandeni

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Supervisor: Dr Emiliano Raffrenato

Co-Supervisors: Prof Christiaan W Cruywagen

Dr Giulia Esposito

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Declaration

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Maria Ndakula Tautiko Shipandeni

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Abstract

Title:	Modulation of starch digestion for productive performance in dairy cows
Candidate:	Maria Ndakula Tautiko Shipandeni
Supervisor:	Dr Emiliano Raffrenato
Co-supervisors:	Prof Christiaan W Cruywagen and Dr Giulia Esposito
Institution:	Department of Animal Sciences, Stellenbosch University
Degree:	PhD in Animal Science

In this thesis, a series of experiments were conducted to ultimately investigate the effects of modulating site of starch digestion by varying ruminal fermentability of various starch sources on feed intake, production and metabolic response of transition cows. The first experiment was performed (Chapter 3) to evaluate the effects of different particle sizes on chemical composition and *in vitro* ruminal starch degradability of cereal grains commonly used in dairy cow diets. Four starch sources (maize 1 and 2, sorghum, barley and wheat) were ground through 1 and 2-mm screens and fractionated by sieving to obtain the following sizes: <250 (very fine), 250-500 (fine), 500-1180 (medium) and 1180-2000 μm (coarse). The generated particle size fractions and unsieved samples were separately analysed for chemical composition and fermented *in vitro* using rumen fluid for 0, 3, 6, 9, 12 and 24 h to determine starch degradability (S_d), and rate of starch degradation (k_d), assuming a first order decay. Particle size affected ($P < 0.0001$) the chemical composition of all grains, with the highest starch in the smallest particles and highest NDF in the largest particles. For all grains, S_d and k_d increased with decreasing particle size. Results from this *in vitro* study suggest that starch digestion could be potentially shifted post-rumen by controlling particle size and reducing the amount fermented in the rumen. In Chapter 4 (experiment 2), we compared two mathematical approaches for determining the rate of starch degradation. The objective was to evaluate the accuracy and precision of the 7 h- k_d 's by comparison with rates obtained using a non-

linear first order decay model as a reference. Higher accuracy and precision were obtained by using a non-linear estimation. There is a need for using a non-linear estimation, using multiple time points or the development of alternative estimations, especially when quantifying rates of starch degradation for high producing cows. Experiment 3 was performed (Chapter 5) to quantify the potential of a starch binding agent (BioProtect™) to reduce *in vitro* rumen starch degradation of cereal grains of varying particles size. Maize and sorghum fractions used in experiment 1 were treated by spraying with BioProtect™ 24 h before *in vitro* fermentation to quantify starch degradability (Sd). Both treated and untreated (no BioProtect™) maize and sorghum samples were fermented *in vitro*. BioProtect™ was effective in decreasing starch degradability for both grains, with effects more pronounced for smaller particle sizes, by reducing Sd 17%-units compared to 7%-units for the largest particles. Simulations with the NDS software indicated that the use of BioProtect™ can reduce rumen starch digestibility, increase rumen starch escape and post rumen starch digestibility. Simulated total tract digestibility was not decreased by the use of BioProtect™ and indicated slightly reduced microbial protein production. Although BioProtect™ showed positive effects on reducing rumen starch degradation, in our simulations, larger particles were more effective at shifting the site of digestion and it could, therefore, be a more cost-effective option for our aim.

Based on the *in vitro* results, two starch sources were selected for further *in vivo* investigation, to study a possible shift in the site of starch digestion. In experiment 4 (Chapter 6) the effects of starch sources and particle sizes on digesta flow, starch digestibility, ruminal fermentation parameters and production performance of dairy cows were investigated. Four ruminally-cannulated multiparous Holstein cows were used in a 4 × 4 Latin square design with a 2 × 2 factorial arrangement of treatments: maize or sorghum (M or S) either finely or coarsely ground (using a 1- or 4-mm screen sieve, F or C). Diets were formulated to contain similar starch concentration. Digesta flow was quantified using the reticular sampling technique, applying the triple-marker method. Dry matter (DM) intake, milk yield and composition were not affected by dietary treatments in exception of MUN. Milk urea nitrogen concentration was lower for cows fed maize diets: 14.36, 14.89, 16.99 and 17.09 mg/dL for MF, MC, SF and SC, respectively. Rumen pH

and reticulum pH were higher for the SC diet (6.20 and 6.56, respectively) when compared to the other treatments. Rumen and reticulum pH were 5.98 and 6.33 for MF, 5.96 and 6.32 for MC, and 5.92 and 6.36 for SF, respectively. Propionate concentration was greater for both maize diets (33.21 and 32.96 vs. 31.22 and 28.68 mM; $P < 0.0001$) and ruminal ammonia N was lower for the fine maize diet compared to the SF and SC diets. Dietary treatments did not affect ($P > 0.05$) organic matter (OM) and NDF intake, nutrient flow of DM, OM and NDF, or ruminal digestibility of OM. Starch from the coarser maize was less ruminally digested (83.76 vs. 88.77% of intake) and had a greater flow to the abomasum when compared to the fine particles (1.04 vs 0.76 kg/d). However, the apparent total-tract digestibility of starch was greater in MF than MC cows (96.29 vs. 87.84%). This study confirms that coarser particles can allow part of starch digestion to be shifted from the rumen to the small intestine, but total tract starch digestibility could be decreased if ruminal digestion is not compensated postruminally.

The objective of experiment 5 (Chapter 7) was to evaluate the effects of starch fermentability of diets fed during the early postpartum (PP) period on feeding behaviour, dry matter intake (DMI), lactation performance and body metabolism of fresh dairy cows. Jersey cows ($n = 117$) were used in a randomized complete block design. Treatment diets were formulated to similar starch concentration, with ground maize (3 or 6-mm screen sieve) as the primary starch source. Treatments were fed as TMR from calving to 30 d PP before switching to a common lactation diet. Throughout the experiment DMI, milk yield and body weight were recorded daily, and milk composition, body condition score (BCS) and blood metabolites were measured weekly. Feeding coarsely ground maize (MC) increased dry matter intake (16.08 vs. 17.13 kg/d) and milk yield (20.41 vs. 21.70 kg/d) compared to finely ground maize (MF). Diet did not affect ($P > 0.05$) eating and rumination time and had no effects on milk composition, but milk lactose was increased in the MC compared to the MF diet (4.70 vs. 4.61%) and milk fat percentage tended to be greater (5.57 vs. 5.27%) in the MF than MC diet. Decreases in BW and BCS were greater in cows fed the MF (39.92 vs 32.24 kg and 0.23 vs. 0.14 units) than in cows fed the MC diet, resulting in increased plasma NEFA concentration (0.71 vs. 0.56 mmol/L) in MF cows. Blood glucose levels were not affected ($P > 0.05$). The increased DMI in cows fed the MC diets could possibly be attributed to reduced production of propionate in the

rumen, resulting from shifting starch digestion postruminally and by the decreased plasma NEFA concentration.

Overall, in conclusion, our results confirm that starch digestibility increases with decreasing particle size, suggesting that starch digestion could be potentially shifted post-rumen by controlling the grain particle size fed and thus reducing the amount fermented in the rumen. BioProtect™, a starch binding agent was effective in reducing *in vitro* rumen starch degradation, with effects more pronounced for smaller particle sizes. Shifting the site of starch digestion postruminally in early postpartum cows increased DMI, milk production and decreased mobilization of body reserves as indicated by the decreased concentration of plasma NEFA. The results of this study apparently support the hepatic oxidation theory of the control of feed intake, particularly during the early postpartum period. More processing alternatives should be investigated to reduce loss of digestibility postruminally for larger particles.

Uittreksel

Titel:	Modulering van styselvertering vir produksieprestasië in melkkoeie
Kandidaat:	Maria Ndakula Tautiko Shipandeni
Studieleier:	Dr.Emiliano Raffrenato
Medestudieleiers:	Prof Christiaan W Cruywagen en Dr Giulia Esposito
Inrigting:	Departement Veekundige Wetenskappe, Universiteit Stellenbosch
Graad:	PhD in Veekunde

Hierdie tesis rapporteer oor 'n reeks eksperimente wat uitgevoer is om die invloed van die modulering van die plek van styselvertering op voerinnamë en produksie- en metaboliese response in oorgangskoeie te ondersoek deur die ruminale fermentasie van verskillende styselbronne te verander. Die eerste proef (Hoofstuk 3) is uitgevoer om die invloed van partikelgrootte op die chemiese samestelling en *in vitro* ruminale styselafbraak van grane, wat tipies in melkbeesrantsoene gebruik word, na te gaan. Vier styselbronne (mielies1 en 2, sorghumgraan, hawer en koring) is deur 1 en 2 mm siwwe gemaal en gefraksioneer deur die gemaalde grane verder deur fyner siwwe te sif om die volgende partikelgroottes te verkry: <250 (baie fyn), 250-500 (fyn), 500-1180 (medium) en 1180-2000 μm (grof). Die resulterende fraksies, asook ongesifte monsters, is apart vir hul chemiese samestelling ontleed en vir 0, 3, 6, 9, 12 en 24 h *in vitro* in gebufferde rumenvloistof gefermenteer om styselgradeerbaarheid (S_d) en tempo van styseldegradering (k_d) te bepaal, met die aanvaarding van 'n eerste-orde afbraak. Partikelgrootte het die chemiese samestelling van al die grane beïnvloed ($P < 0.0001$) en die hoogste styselinhoud is in die fynste partikels gevind en die hoogste NDF-inhoud in die grofste partikels. Vir al die grane het S_d en k_d toegeneem namate partikelgrootte afgeneem het. Resultate van hierdie *in vitro*-studie dui daarop dat styselvertering potensieel na die post-ruminale verteringskanaal verskuif kan word deur partikelgrootte

te beheer en die hoeveelheid stysel wat in die rumen fermeteer word te verlaag. In Hoofstuk 4 (Eksperiment 2), is twee wiskundige benaderings vergelyk waarmee die tempo van styselvertering bepaal word. Die doel was om die akkuraatheid en presisie van die 7 h k_d waardes te vergelyk met beramings wat verkry is met 'n nie-lineêre eerste-ordemodel as verwysing. 'n Hoër mate van akkuraatheid en presisie is met die nie-lineêre beraming verkry. Daar bestaan 'n behoefte aan die gebruik van 'n nie-lineêre beraming met veelvuldige tydintervalle, of die ontwikkeling van alternatiewe beramings, veral wanneer die tempo's van styselvertering vir hoogproduserende melkkoeie gekwantifiseer word. Eksperiment 3 (Hoofstuk 5) is gedoen om die potensiaal van 'n styselbindingsagent (BioProtect™) te kwantifiseer om *in vitro* styselafbraak van verskillende grane en variërende partikelgroottes te verlaag. Die mielie- en sorghumfraksies wat in Eksperiment 1 gebruik is, is 24 h voor *in vitro*-fermentasie met BioProtect™ behandel om styselafbraak (Sd) te kwantifiseer. Beide behandelde en onbehandelde monsters van mielies en sorghumgraan is *in vitro* gefermenteer. BioProtect™ was doeltreffend om styselafbraak in beide grane te verlaag en die invloed was groter met die kleiner partikels waar Sd met 17 persentasie-eenhede verlaag is in vergelyking met 7 persentasie-eenhede vir die grootste partikels. Simulasies met behulp van die NDS sagteware het aangetoon dat die gebruik van BioProtect™ ruminale styselvertering kan verlaag en terselfdertyd die ruminale verbyvloeiwaarde en post-ruminale styselvertering kan verhoog. Gesimuleerde totale-kanaal verteerbaarheid is nie deur BioProtect™ verlaag nie en het 'n geringe verlaging in die produksie van mikrobiële proteïene aangedui. Hoewel BioProtect™ positiewe resultate getoon het om ruminale styselafbraak te verlaag, het simulasies aangedui dat groter partikels meer doeltreffend was om die plek van vertering te skuif en kan dit dus moontlik 'n meer koste-effektiewe opsie wees om die doel van die studie te bereik.

Gebaseer op die *in vitro*-resultate, is twee styselbronne geselekteer vir verdere *in vivo*-ondersoeke om die moontlike verskuiwing van die plek van styselvertering te bestudeer. In Eksperiment 4 (Hoofstuk 6) is die invloed van styselbron en partikelgrootte op die vloeï van digesta, styselverteerbaarheid, ruminale fermentasieparameters en produksierespons van melkkoeie ondersoek. Vier rumengekannuleerde Holsteinkoeie is in 'n 4 x 4 Latynse vierkantontwerp, met 'n 2 x 2 faktoriale indeling van behandelings,

gebruik: mielies of sorghum (M of S), fyn of grofgemaal (deur 'n 1 of 4 mm sif, F of C). Diëte is geformuleer om dieselfde styselinhoud te hê. Digestavloei is gekwantifiseer deur van die retikulêre monsternemingstegniek gebruik te maak, met die toepassing van die trippelmerkermetode. Droëmateriaal (DM) inname, melkproduksie en melksamestelling is nie deur behandelings beïnvloed nie, behalwe vir MUN. Die melk-ureumstikstofinhoud was laer vir koeie op die mieliediëte: 14.36, 14.89, 16.99, en 17.09 mg/dL vir MF, MC, SF en SC, onderskeidelik. Rumen pH en retikulum pH was hoër vir die SC dieet (6.20 en 6.56, onderskeidelik) in vergelyking met die ander behandelings. Rumen- en retikulum pH was 5.98 en 6.33 vir MF, 5.96 en 6.32 vir MC en 5.92 en 6.36 vir SF, onderskeidelik. Propionaatkonsentrasies was hoër vir beide mieliediëte (33.21 en 32.96 vs. 31.22 en 28.68 mM; $P < 0.0001$) en rumen-ammoniak-N was laer vir die fyn mieliedieet in vergelyking met die SF en SC diëte. Dieetbehandelings het nie organiese materiaal (OM) en NDF-inname, nutriëntvloei van DM, OM en NDF, of ruminale OM-verteerbaarheid beïnvloed nie. Stysel van die grower mielies het 'n laer rumenverteerbaarheid getoon (83.76 vs. 88.77% van inname) en het 'n groter vloei na die abomasum gehad in vergelyking met die fyn partikels (1.04 vs. 0.76 kg/d). Die skynbare totalekanaalverteerbaarheid van stysel was egter hoër in die MF as in die MC koeie (96.29 vs. 87.84%). Hierdie studie bevestig dat growwer partikels die vermoë het om styselvertering gedeeltelik vanaf die rumen na die laer spysverteringskanaal te verskuif. Totalekanaal-styselverteerbaarheid kan egter verlaag word indien daar nie post-ruminaal vir ruminale styselvertering gekompenseer word nie.

Die doel van Eksperiment 5 (Hoofstuk 7) was om die invloed van stysel-fermenteerbaarheid in vroeë-laktasiediëte vir melkkoeie op voedingsgedrag, droëmateriaalinname (DMI), melkproduksierespons en liggaamsmetabolisme na te gaan. Jerseykoeie ($n = 117$) is in 'n gerandomiseerde blokontwerp gebruik. Behandelingsdiëte is geformuleer om dieselfde styselvlakke te bevat, met gemaalde mielies (3 of 6 mm sif) as die primêre styselbron. Die behandelingsdiëte is in die vorm van 'n TGR aangebied, vanaf kalwing tot 30 dae na kalwing wanneer die koeie weer teruggeplaas is op die normale laktasiedieet. Gedurende die proefperiode is melkproduksie en liggaamsmassa (LM) daaglik aangeteken, terwyl melksamestelling, liggaamskondisie (BCS) en bloedmetaboliete weekliks bepaal is. Die voeding van grofgemaalde mielies (MC) het DMI

verhoog (16.08 vs. 17.13 kg/d), asook melkproduksie (20.41 vs. 21.07 kg/d) verhoog in vergelyking met fyn mielies (FM). Dieet het nie vreet- en herkoutyd beïnvloed nie, terwyl melksamestelling ook nie beïnvloed is nie, behalwe in die geval van melklaktose wat hoër was in die MC diet in vergelyking met die MF diet (4.70 vs. 4.61%) en die bottervetinhoud wat geneig het om hoër te wees in die MF diet teenoor die MC diet (5.57 vs. 5.27%). Die afname in LM en BCS was groter in koeie wat die MF dieet ontvang het as in dié op die MC dieet (39.92 vs 32.24 kg en 0.23 vs. 0.14 eenhede), wat gelei het tot verhoogde NEFA konsentrasies (0.71 vs. 0.56 mmol/L) in die MF koeie. Bloedglukosekonsentrasies is nie beïnvloed nie. Die verhoogde DMI in koeie wat die MC diëte ontvang het kan moontlik toegeskryf word aan die verlaagde propionaatproduksie in die rumen, wat die resultaat was van 'n verskuiwing van styselvertering na die dunderm en ook as gevolg van die daling in NEFA konsentrasies.

In die algemeen bevestig die huidige studieresultate dat styselvertering verhoog namate partikelgrootte afneem, wat daarop dui dat styselvertering na die laer spysverteringskanaal verskuif kan word deur die partikelgrootte van grane te beheer en sodoende die hoeveelheid stysel wat in die rumen fermenter word te verlaag. BioProtect™, 'n styselbindingsagent, was doeltreffend om *in vitro* styselvertering te verlaag en die effek was groter in die geval van die kleiner partikelgroottes. Deur styselvertering in vroeëlaktasiekoeie post-ruminaal te verskuif, het die DMI en melkproduksie verhoog, terwyl die mobilisering van liggaamsreserwes verlaag het, soos aangedui deur die verlaagde plasma NEFA konsentrasies. Resultate van hierdie studie ondersteun skynbaar ook die hepatiese oksidasieteorie van voerinnamebeheer, veral gedurende die vroeë laktasieperiode. Meer prosesseringsalternatiewe behoort ondersoek te word om om die postruminale verlies van styselvertering van groter partikels te verlaag.

Dedication

To those who are inspired

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*We can help each other fly and reach greater heights.
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List of Abbreviations

°C	Degree celsius
ADF	Acid detergent fibre
ADL	Acid detergent lignin
AOAC	Association of Official Analytical Chemists
ATP	Adenosine triphosphate
BCS	Body condition score
BW	Body weight
BHBA	β-hydroxybutyric acid
CP	Crude protein
CF	Crude fat
CNCPS	Cornell net carbohydrate and protein system
Co-EDTA	Cobalt- Ethylenediaminetetraacetic acid
d	Day
DM	Dry matter
DIM	Days in milk
DMI	Dry matter intake
ECM	Energy corrected milk
EE	Ether extract
FP	Fluid phase
GC	Gas chromatography
GIT	Gastro-intestinal tract
GLM	Generalised linear model
GMPS	Geometric mean particle size
HOT	Hepatic oxidation theory
TG	Triglyceride
iNDF	Indigestible NDF
ivSd	<i>in vitro</i> starch degradability

K _d	Rate of starch degradation
LSM	Least squares means
LP	Large particle phase
ME	Metabolizable Energy
MFD	Milk fat depression
MP	Metabolizable protein
mPS	Mean particle size
MUN	Milk urea nitrogen
MY	Milk yield
NDF	Neutral detergent fibre
NEB	Negative energy balance
NEFA	Non-esterified fatty acids
NE _L	Net energy for lactation
NFC	Non fibre carbohydrates
NGMPS	Nominal geometric mean particle size
NH ₃	Ammonia
NRC	National Research Council
pH	Potential hydrogen
RT	Rumination time
SAS	Statistical Analysis System
SCC	Somatic cell count
SD	Starch degradability
SEM	Standard error of least square means
SP	Small particle phase
TMR	Total mixed ration
TTSD	Total tract starch digestibility
VFA	Volatile fatty acids
YB	Ytterbium

Notes

The language and style used in this thesis are in accordance with the requirements of the Journal of Dairy Science, with modification to the spelling of words used in South Africa. This thesis, apart from the General introduction and the Overall Conclusions chapters (i.e. 1 and 8), represents a compilation of manuscripts where each chapter is an individual article, therefore some repetition between chapters has been inevitable. All literature were referenced according to guidelines of the Journal of Dairy Science.

CHAPTER 1

General Introduction

Intensive genetic selection for milk production over the last three to four decades has increased milk yield of dairy cows, resulting in increased nutrient requirements, especially energy. To manifest the genetic potential of high-yielding dairy cows, they are fed substantial amounts of cereal grains (maize, sorghum, wheat and barley) to increase the energy density of the diet in the form of starch. The starch content of cereal grains varies greatly, ranging from 58 (oats) to 77% (maize), affected by the variety, agronomic practices and growing conditions (Huntington, 1997). The optimum dietary starch content for lactating dairy cows is still not well defined, but is suggested to range from 25 to 30% (dry matter (DM) basis; Allen and Piantoni, 2014), depending primarily on milk yield, the stage of lactation and forage content of the diet (Lean et al., 2014).

Starch is digested either in the rumen or post-ruinally, with the rumen being the major site of starch digestion (Theurer, 1986). In the rumen, starch is fermented by rumen microbes to yield propionate, which is used as a precursor for glucose (Drackley et al., 2001; Reynolds et al., 2003; Aschenbach et al., 2010). Unfermented starch flowing into the small intestine is digested by pancreatic amylase directly into glucose, while some starch may be fermented in the hindgut to yield propionate or is excreted in the faeces. Ruminal fermentability of starch is highly variable, ranging from 22 to 94% (Moharrery et al., 2014), depending on intrinsic factors, primarily the grain/endosperm type (i.e. starch-protein matrix) and processing, as well as by extrinsic factors such as ration composition and animal factors (e.g. starch intake) as reviewed recently by Giuberti et al. (2014). On the other hand, the digestion of starch within the small intestine is limited (Reynolds, 2006; Ferraretto et al., 2013; Mills et al., 2017), but the limiting factors are still not well defined (Owens et al., 1986; Huntington, 1997; Mills et al., 2017). The rate, extent and site of starch digestion have been studied for decades using different techniques. Although *in vivo* studies represent the true biological method to define the dynamics of starch digestion, *in vitro* techniques are widely adopted (Huhtanen and Sveinbjörnsson, 2006; Sveinbjörnsson et al., 2007; Giuberti et al., 2014), and can provide reliable *in vivo* reference data useful in feed formulation models to accurately predict the site and extent

of starch digestion, and hence the animal performance (Offner and Sauvant, 2004; Patton et al., 2012; Moharrery et al., 2014; Mills et al., 2017). Moreover, rates of starch degradation determined *in vitro* are incorporated into ration formulation programs to predict ruminal starch digestibility.

Altering the concentration and ruminal fermentability of starch affects total digestibility of starch, ruminal pH and fibre digestibility, and the type, amount, and temporal absorption of end products (e.g. acetate, propionate, lactate, glucose) available to the cow (Allen, 2000). This affects lactational and reproductive performance by affecting energy intake and partitioning as well as absorbed protein (Allen et al., 2009). In this context, Allen et al. (2009) proposed the hepatic oxidation theory (HOT), which suggests that propionate derived from rumen fermentation and non-esterified fatty acids (NEFA) from mobilization of body reserves are the main regulator of dry matter intake (DMI) in dairy cows. Greater ruminal fermentation of starch increases production of propionate in the rumen and decreases feed intake, likely due to hypophagic signals from increased hepatic oxidation of fuels (Allen, 2000). The hypophagic effects of propionate have been supported by intraruminal propionic acid infusion studies (Oba and Allen, 2003; Stocks and Allen, 2012, 2013; Gualdrón-Duarte and Allen, 2018; Maldini and Allen, 2018). According to the HOT, the physiological state determines the effects of starch fermentability on DMI, production and reproductive responses (Allen, 2000; Allen et al., 2009). During the transition period (i.e. from 3 weeks before to 3 weeks after calving), particularly immediately after calving, cows are in a lipolytic state with an elevated concentration of plasma NEFA, which intensifies the hypophagic effects of propionate (Oba and Allen, 2003; Allen et al., 2009; Stocks and Allen, 2012, 2013).

Typically, transition cows undergo enormous physiological and metabolic challenges and stress (Bell, 1995; Grummer, 1995; Drackley, 1999; Sordillo and Raphael, 2013). These cows experience a period of considerable increase of energy demand, dramatic reduction in DMI ($\geq 30\%$) and negative energy balance (NEB), which is associated with metabolic and health problems, reduction in milk yield and reproductive performance (Goff and Horst, 1997; Drackley, 1999; Butler, 2003; Mulligan and Doherty, 2008; LeBlanc, 2010; Esposito et al., 2014). Moreover, disturbances in the HOT mechanism and NEB delay the onset of first ovulation after parturition and affect oocyte

quality and subsequently fertility. Inclusively, these problems increase the incidences of involuntary culling (Esposito et al., 2014), affecting the profitability of dairy farms.

Overall, the mechanisms controlling feed intake in transition cows are not well understood (Ingvarsen and Andersen, 2000) and remain a challenge. Therefore, nutritional strategies immediately postpartum to support metabolic adaptations and optimize DMI, production and reproduction performance in transition cows are needed. The hepatic oxidation theory suggests that the site of starch digestion can modulate the occurrences of problems encountered by transition cows, but with variability of effects across lactation (Allen et al., 2009). Larsen et al. (2009) also suggested that feeding rations that partly shift the site of starch digestion from the rumen to the small intestine is an attractive strategy to overcome some of the nutritional shortcomings associated with meeting the nutrient needs of transition cows. Several studies have examined various ways to modulate the rumen degradability of starch (Huntington et al., 2006; Reynolds, 2006), with mechanical grain processing (grinding) being the most common and less expensive way of shifting site of starch digestion (Knowlton et al., 1998b; Yu et al., 1998; San Emeterio et al., 2000; Callison et al., 2001; Rémond et al., 2004; Larsen et al., 2009; Fredin et al., 2015). While the energetic efficiency of starch utilization in the small intestine is high (Owens et al., 1986; Reynolds et al., 2001; Huntington et al., 2006), extensive reviews by Nocek and Tamminga (1991) and Reynolds (2006) have reported that the production responses of dairy cows to the site of starch digestion are equivocal. Currently, there is not enough evidence to support the HOT as previous studies have been contradictory, in fact milk production increases with greater ruminal starch fermentability (McCarthy et al., 2015; Albornoz and Allen, 2018). More propionate to glucose enhances both protein efficiency (higher microbial protein yield and milk protein) and yield of milk (Theurer et al., 1999), and glucose originating from ruminal escape starch can be used for milk synthesis (McCarthy et al., 1989). Contrary, starch or glucose infusion studies have shown how glucose from starch digested in the small intestine would be primarily used for body tissue protein and fat deposition rather than milk production (Reynolds et al., 2001), or it may be oxidized to CO₂ (Knowlton et al., 1998a). Results from reproductive studies are also controversial (Gong et al., 2002; Knegsel, 2007; Garnsworthy et al., 2009). Moreover, most of the studies were conducted outside of the transition period, the

delicate phase, which according to the HOT is characterized by relevant physiological changes that affect DMI, milk yield and quality and reproductive responses. All these contrasting results would be only partially justified by the HOT and the changing physiological response of a lactating cow that would confirm the fact that glucose from small intestine digestion would be used for production needs in animals having a high production demand for energy, such as early lactation dairy cows (Allen et al., 2009).

The overall aim of this thesis was to investigate potential practical ways of modulating site of starch digestion in transition cows, and its effects on various animal parameters.

The specific objectives of the study were to:

- i. Evaluate the effects of different particle sizes on chemical composition and *in vitro* ruminal starch degradability of cereal grains commonly used in dairy cow diets.
- ii. Compare mathematical approaches used for determining the rate of starch digestion *in vitro*.
- iii. Quantify the potential of a starch binding agent (BioProtect™) to reduce *in vitro* rumen starch degradation of cereal grains varying in particles size.
- iv. Evaluate the effects of starch sources varying in particle sizes on digesta flow, starch digestibility, ruminal fermentation parameters, and production performance of dairy cows.
- v. Evaluate the effects of rumen fermentability of starch on feeding behavior, dry matter intake, productive and metabolic response of early lactating dairy cows.

It was hypothesized that by partially shifting the site of starch digestion from the rumen to the small intestine would reduce the negative effects (DMI; productive and reproductive performance) associated with high starch digestion in the rumen in transition cows.

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CHAPTER 2

LITERATURE REVIEW

2.1. Abstract

During the past few years, there has been a surge of research interest in dietary starch, both in terms of content and fermentability, which is a primary source of energy for dairy cows. This has been motivated by discussions related to carbohydrate formulation of transition diets and potential interactions with DMI, coupled with perpetual challenges and stress of the transition cow. Typically, dairy cows are fed substantial amounts of starch (supplied mainly by cereal grains) to meet the energy demand and support the genetic merit for high milk production. Greater ruminal starch fermentation increases production of propionate, which according to the hepatic oxidation theory (HOT) decreases feed intake. The hypophagic effects are more pronounced in early lactation when cows are in the lipolytic state. Generally, transition cows are characterised mainly by negative energy balance (NEB), decreased feed intake and perturbed immune function that predispose cows to periparturient diseases, coupled with reduced production and reproduction performance. This review discusses the impact of starch sources, concentration and its site of digestion on dry matter intake (DMI) and performance of transition cows, in order to evaluate the optimal strategy to reduce its risks. Currently, there is insufficient evidence to support the HOT as previous studies have been contradictory. Moreover, the effects of starch content and fermentability on lactational performance, metabolic status and reproduction of transition cows are still inconclusive. Research on this critical phase of the production cycle of dairy cows is limited. Therefore, further research is necessary in this area to better understand the interaction between starch content and fermentability during the transition period and reduction in DMI. This will enable better formulation of diets, which will attenuate the abrupt changes in nutrient supply, maximise DMI and reduce adverse effects of NEB, hence profitability of the dairy farms.

2.2. Introduction

The transition period (also referred to as the periparturient period) is generally described as the three weeks before to three weeks after calving (Drackley, 1999), whereby the production cycle of the cow shifts from the gestational non-lactating state to the post-parturient status with the onset of milk synthesis and secretion. This period has troubled the dairy industry for decades, despite the prodigious output of research on the physiology, adaptations, nutrition and management of transition cows (Overton and Waldron, 2004; Ingvarlsen, 2006; Van Saun and Sniffen, 2014). Numerous challenges and stress with, possibly, long-term impacts on the health, reproductive performance, milk yield and therefore the profitability of the dairy industry, occur during this phase. It is during this period that energy demand of the cow increases tremendously (Reynolds et al., 2003), yet dry matter intake (DMI) is greatly decreased (Bell, 1995). This results in a negative energy balance (NEB), intense body fat mobilization and, subsequently, increased risk of metabolic disorders and infectious diseases (Grummer, 1993; Mulligan and Doherty, 2008) and impaired reproduction performance (Butler, 2000; Esposito et al., 2014; Santos and Ribeiro, 2014), culminating in economic losses. Unfortunately, the genetic selection for milk yield and solids over the past 50 years has intensified the requirements for lactogenesis and galactopoiesis (Bell, 1995), exacerbating NEB during early lactation.

There has been intense interest in understanding the regulation of feed intake in dairy cows, but the mechanism of decreased DMI during the transition period has remained elusive as reviewed by Ingvarlsen and Andersen (2000), with new insights discussed more recently (Allen et al., 2009; Stocks and Allen, 2012, 2013, Allen and Piantoni, 2013, 2014). In intensive systems, high producing dairy cows are fed high concentrations of starch to maximize dietary energy intake to allow the genetic potential for milk energy yield to be realized with minimal negative effects on health and reproduction. Starch is digested mainly in the rumen to yield propionate as end product. Small intestine (SI) starch digestion yield glucose as end product. According to the hepatic oxidation theory (HOT), feed intake, especially in the transition period, would be controlled by oxidation of fuels in the liver, mainly propionate and non-esterified fatty acids

(NEFA) (Allen et al., 2009). Increased oxidation of fuels in the liver can increase the energy status of the liver and ultimately signals the brain to decrease feed intake (Allen et al., 2009). Intraruminal and abomasally continuous infusion of propionic acid is reported to decrease DMI of cows in the postpartum period (Oba and Allen, 2003a; Stocks and Allen, 2013; Gualdrón-Duarte and Allen, 2018). This is presumably because of the ability of propionic acid to stimulate hepatic oxidation (Gualdrón-Duarte and Allen, 2018). It has also been reported that the hypophagic effects of propionic acid is more pronounced in early lactation than mid lactation (Oba and Allen, 2003a), when cows are in a lipolytic state (Stocks and Allen, 2012). According to the HOT, the physiological state of the ruminant determines the extent of the effects that starch content and fermentability have on DMI, productive and reproductive responses (Allen et al., 2009). This proposed theory is, however, still under investigation.

The objective of this review is to describe the impact of starch sources, concentration and its site of digestion on DMI and performance of transition cows, in order to evaluate the optimal strategy to reduce its risks.

2.3 Adaptation and major challenges of transition cows

The transition period is the most tumultuous and challenging phase of the production cycle of dairy cows, as the management thereof plays an important role on subsequent health status, production, reproduction of the cow and hence on the profitability of a dairy farm. The production cycle shifts from the gestation non-lactating to the lactating non-gestation state. This period encompasses numerous challenges and stress factors as it coincides with major endocrine, metabolic, physiological, immunological, nutritional and social changes (Bell, 1995; Grummer, 1995; Drackley, 1999; Ingvarlsen and Andersen, 2000; Drackley et al., 2005; Mulligan and Doherty, 2008; Roche et al., 2013; Sordillo and Raphael, 2013).

Typically, DMI starts reducing ($\geq 30\%$) at approximately three weeks preceding parturition, with a dramatic decline in the last seven days (Bertics et al., 1992; Bell, 1995; Hayirli et al., 2002) (Figure 2.1), but yet the energy demand increases considerably to support a great increase of fetal growth, mammogenesis, and lactogenesis (Grummer, 1995; Ingvarlsen and Andersen, 2000; Overton and Waldron, 2004; Ingvarlsen, 2006).

The DMI increases postpartum, but the rate and level of increase vary considerably and lags behind the demand of lactation (Ingvartsen and Andersen, 2000). The mechanism for depressed feed intake during the transition period has been debated and has remained elusive (Ingvartsen and Andersen, 2000; Grummer et al., 2004; Allen et al., 2009; Allen and Piantoni, 2013). Several mechanisms and theories have been proposed over the time, including physical (gut distention) (Forbes, 2007), metabolic, hormonal and endocrine factors, as well as environmental and management factors, as intensively discussed by Ingvartsen and Andersen (2000). The latest theory is the hepatic oxidation theory (HOT) proposed and discussed by Allen (Allen, 2000; Allen et al. 2009). The underlying cause of the decrease in feed intake during the transition period is, however, still under investigation, and seems to be complex and multifactorial.

Grummer (1995) illustrated the relationship between energy requirement, intake and balance of the entire transition period. Typically, transition cows experience a period of negative energy balance (NEB) as a result of the rapid increase of energy requirements and a simultaneous marked decrease in DMI. This sudden change in the energy demand versus supply (energy imbalance) is inevitable and requires a dramatic shift in energy metabolism and partitioning involving aspects of homeostatic and homeorhetic controls (Bauman and Currie, 1980; Lucy, 2008) in order to support the increased demand for fetal growth and direct nutrients to the mammary gland to support lactogenesis (Grummer, 1995; Ingvartsen, 2006). The metabolic adaptations that support this abrupt change include increased hepatic gluconeogenesis (Reynolds et al., 2003; Aschenbach et al., 2010), increased mobilization of body fat (Pettersen et al., 1994) and decreased use of glucose for fuel by peripheral tissues (primarily skeletal muscles) (Bell and Bauman, 1997; Hayirli, 2006; De Koster and Opsomer, 2013). As a result, glucose is spared for use by the gravid uterus and lactating mammary gland. These homeorhetic adaptations are coordinated across various organs and tissues (such as the liver, the mammary gland, gut and adipose, muscular and hepatic tissues) and require regulations of various biological processes as discussed by Ingvartsen (2006). Metabolic changes occur in all cows, including those that are well fed, however, cows adapt differently to the metabolic challenges despite similar performance levels, feeding and housing conditions (Weber et al., 2013).

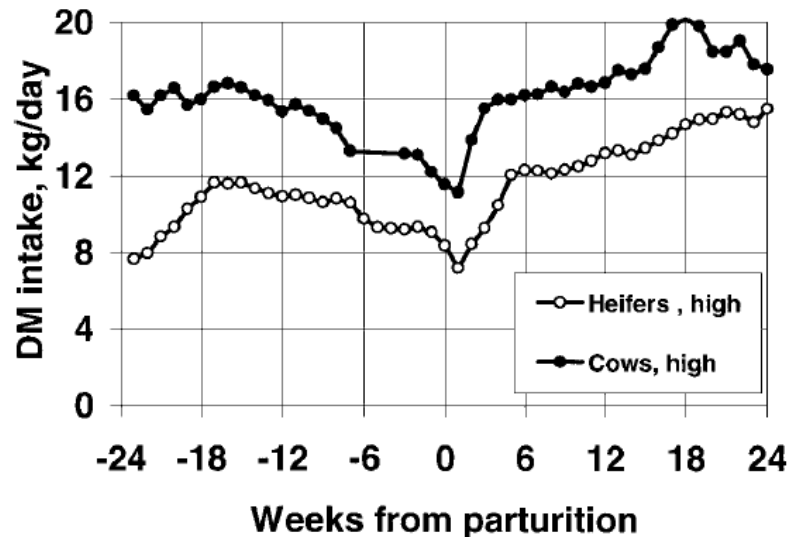


Figure 2. 1. The pattern of voluntary dry matter intake during the transition period in heifers and cows (from: Ingvarsen and Andersen, 2000).

Typically, the NEB begins a few days prepartum as the DMI lags behind the energy required, reach its nadir usually in two weeks postpartum (Butler, 2000) and may last until approximately six to nine weeks postpartum (Grummer et al., 2010). This state of NEB results in mobilization of body fat, which circulates as non-esterified fatty acids (NEFA). The NEFA are taken up by the liver, where some are oxidized or re-esterified into triglycerides; these are either exported as very low density lipoproteins, which go to the mammary gland for milk fat production and to other tissues to serve as an energy source, or stored in the liver (Drackley et al., 2001). The fate of mobilized body fat in lactating dairy cows has been extensively discussed and illustrated (Drackley, 1999; Drackley et al., 2001). Although mobilization of body fat is inevitable and accounts for the energy deficit in early lactation, hence providing energy needed to support milk production. Intense body fat mobilization during NEB is associated with markedly elevated concentrations of plasma NEFA, β -hydroxybutyrate (BHBA) and low concentrations of insulin and insulin-like growth factor I (IGF-I) (Bell, 1995; Drackley et al., 2001; Bobe et al., 2004; Adewuyi et al., 2005; Hayirli, 2006; Weber et al., 2013). A low concentration of insulin in the blood, which results in decreased adipose tissue lipogenesis and an increase in lipolysis, further increases NEFA in the blood. High levels of NEFA and BHBA

concentrations in the blood are indicative of lipid mobilization and fatty acid oxidation, respectively (Wathes et al., 2007; LeBlanc, 2010).

The duration and depth of NEB vary among cows which are primarily related to DMI differences, the rate of DMI increase during early lactation as well as the genetic merit and hence milk yield of the cow, pre-calving body condition and diet (Wathes et al., 2007). It has been observed that cows with high body condition score (BCS) at prepartum mobilize more body reserves (Janovick and Drackley, 2010). Fat mobilization varies greatly among high yielding dairy cows (Weber et al., 2013), with some cows able to cope with metabolic stress better than others (Herdt, 2000). The severity and magnitude of NEB is closely associated with immune suppression, increased risk of metabolic disorders and health problems (Ingvarsen, 2006; Mulligan and Doherty, 2008; LeBlanc, 2010; Ospina et al., 2010; Roche et al., 2013; Santos and Ribeiro, 2014; Sundrum, 2015). These health problems occur primarily within the first 2 weeks of lactation (Goff and Horst, 1997; Drackley, 1999). Despite the tight homeostatic controls and homeorhetic adjustments to cope with the changes in metabolism to support milk production up to 45-71% of dairy cows across different levels of milk production, breeds and management systems, succumb to metabolic disorders and infectious diseases (Santos and Ribeiro, 2014). Cows that are over-conditioned at the onset of lactation are at greater risk of developing metabolic diseases (Collard et al., 2000; Kim and Suh, 2003). These disorders include ketosis (Grummer, 1993; Drackley, 1999), fatty liver (Bobe et al., 2004), hypocalcaemia (milk fever) (Goff, 2008), displaced abomasum (Shaver, 1997; LeBlanc et al., 2005), retained placenta and metritis (Huzzey et al., 2007; Melendez et al., 2009). Moreover, cows become more susceptible to infectious diseases such as mastitis (Ingvarsen et al., 2003), locomotive problems (Collard et al., 2000) and experience impaired reproductive performance (Butler, 2003; Walsh et al., 2011; Santos and Ribeiro, 2014). Transition disorders and diseases have a complex interrelationship, and should not be considered in isolation to minimize their negative impacts (Sundrum, 2015). They directly or indirectly result in significant economic losses through increased culling and mortality risks, reproduction losses, milk losses and milk composition alteration in addition to veterinary costs.

Besides reduced feed intake, negative energy balance (NEB), insulin resistance in peripheral tissues, lipolysis, weight loss in early lactation and hypocalcaemia in the few days postcalving, dairy cows also experience a period of reduced immune function for 1 to 2 weeks before and 2 to 3 weeks after calving (Goff and Horst, 1997; LeBlanc, 2010). Periparturient immunosuppression increases the susceptibility of a cow and severity of metabolic disorders and infectious diseases (Mallard et al., 1998; Drackley, 1999). Immunosuppression and dysfunctional inflammatory response during the transition period have been extensively reviewed recently (Sordillo and Raphael, 2013; Esposito et al., 2014; Sordillo and Mavangira, 2014; Sundrum, 2015; Sordillo, 2016).

2.4 Sources and content of starch in diets of transition cows

Starch is the major source of energy in diets of dairy cows, supplied primarily by cereal grains such as maize, sorghum, wheat, barley and oats. Morphologically, cereal grains consist of the outer layer known as the pericarp (seed coat or bran) that account for about 3 to 8% of the grain weight in maize and sorghum but approximately 25% in oats and barley; the endosperm (the largest proportion, 60-90%) and the germ (embryo) (Kotarski et al., 1992; Huntington, 1997; Corona et al., 2006). Architecturally, starch is made up of polymers of glucose, amylose and amylopectin (Tester et al., 2004), embedded in starch granules in the endosperm, with variation in solubility and digestibility (Kotarski et al., 1992). Most cereal grain starch granules consist of ~25% of amylose and ~75% of amylopectin, with variation in composition, shape and size depending on the botanical origin (Kotarski et al., 1992; Tester et al., 2004; Svihus et al., 2005).

Typically, cereal grains are supplemented to forage to increase the energy density of the diet, to maximize energy intake of high yielding dairy cows, to provide glucose precursors, mainly propionate, and to decrease the filling effects of forage. The average content of starch varies greatly among cereals, with wheat containing 77%, maize and sorghum 72%, and barley and oats 58% on a DM basis, depending on the variety, agronomic practices and growing conditions (Huntington, 1997). Apart from cereal grains, cereal silages also provide starch, varying from <20 to >65% of DM in high moisture maize and whole plant maize silage (Hoffman et al., 2011; Ferraretto et al., 2015), depending

mainly on the stage of plant maturity at harvest (Johnson et al., 1999). Based on the NRC (2001) calculations, starch from maize grain and maize silage accounts for approximately 75% and 50% of its energy value, respectively, hence the importance in the diet of high energy demanding dairy cows. Besides providing energy to the cow, starch in cereals also serves as a source of energy for microbial growth and, therefore, it has a great impact on metabolizable protein as well.

Table 2. 1. Average starch content, digestibility and degradation rate of cereal grains commonly used in dairy cow diets (%).

	Maize	Sorghum	Barley	Wheat
Starch (%) ^{1,2}	72.0 - 76.0	72.0 - 75.0	57.0 - 74.0	67 - 77.0
Rumen digestibility (% of intake) ^{2*}	72.0 - 89.9	60.0 - 78.4	80.7 - 84.6	88.1 - 88.3
Post rumen digestibility (% of starch intake) ^{2*}	6.3 - 44.0	15.4 - 26.1	13.6 - 13.7	10.0
Post rumen digestibility (% of starch entering) ^{2*}	67.8 - 92.6	46.1 - 89.9	75.2- 88.0	85.4 - 88.2
Total tract digestibility ^{2*}	91.2 - 98.9	87.2 - 98.0	94.3 - 98.2	98.2 - 98.6
<i>Degradation rate^{3,4}</i>				
<i>a</i> (%)	23.6	27.7	51.5	60.4
<i>c</i> (% h ⁻¹)	5.9	4.2	35.0	32.9
ED (%)	59.7	60.3	91.3	93.9

*Variability is explained by grain processing (grinding, rolling, flaking). *a* = fraction of starch disappearing immediately, *c* = fractional degradation rate of potentially degradable starch. ED = effective digestibility (%; *passage rate 0.06 h⁻¹*). Degradation rate based on untreated grains. Adapted from ¹Herrera-Saldana et al., 1990;

²Huntington, 1997; ³Offner et al., 2003; ⁴Zebeli et al., 2010).

Although the concept of increasing energy density of diets during the transition period has been in existence for decades (Grummer, 1995; Rabelo et al., 2003; Overton and Waldron, 2004), the optimum starch content and rumen digestibility for transition cows is still not well defined, possibly because of contradictory findings (Rabelo et al., 2003, 2005; Overton and Waldron, 2004; McCarthy et al., 2015a). However, the starch content in diets of transition cows ranges from 15 - 18% prepartum to 20 - 26% postpartum on a DM basis (Lean et al., 2014; Van Saun and Sniffen, 2014), depending

on the NDF and forage NDF content of the diet (Lean et al., 2014). Table 2.1 indicates the variation in starch content, digestibility and degradation rate of commonly used cereal grains in the diets of dairy cows.

2.5 Fate of starch in the digestive system of dairy cows

2.5.1 Ruminal starch: digestion, end-products and limitations

The rumen is the major site of starch digestion (Theurer, 1986). In the rumen, starch is fermented by a diverse species of rumen microbes, mainly amylolytic bacteria to yield mainly propionate, as a major source of energy, microbial protein, as well as CO₂, methane and heat (Huntington, 1997). Rumen amylolytic bacteria adhere and colonize grain particles to produce enzymes that hydrolyze the amylose and amylopectin glycosidic bonds resulting in different oligosaccharides (McAllister et al., 1994; Tester et al., 2004). Although each amylolytic bacteria is capable of digesting starch, they are not equipped with a complete array of enzymes necessary to digest the entire grain. Therefore, these bacteria form a consortium and integrate to attain rapid and maximum digestion of starch as they produce different amylolytic enzymes needed to convert starch to monosaccharides (Kotarski et al., 1992; Tester et al., 2004). Besides bacteria, rumen protozoa also digest large amounts of starch by ingesting starch granules, and fungi aid in bacterial attachment by creating lesions on the surface of the grain (Kotarski et al., 1992; Mendoza et al., 1993; McAllister et al., 1994; Huntington, 1997). Protozoa are found in large quantities ($\geq 8 \times 10^5/\text{mL}$) in cattle fed high-grain diets (Towne et al., 1990; Nagaraja et al., 1992; Mendoza et al., 1993; Franzolin and Dehority, 1996; Hristov et al., 2001) unless the increase in acid production caused by the high grain level reduces the rumen pH to ≤ 5.5 . Depending on the rate of feed intake, rate of passage, and salivary production, this will reduce protozoa numbers (Hoover and Miller, 1991; Franzolin and Dehority, 1996). Under conditions of ruminal pH ≥ 5.5 , about 20 to 45% of the amylolytic activity in the rumen has been attributed to protozoa (McAllister and Cheng, 1996). Ruminal protozoa are known to influence the rate of ruminal starch digestion by engulfing and digesting starch granules and soluble sugars, thereby decreasing the amount of

substrate available to bacteria (Mendoza et al., 1993) and by predating on amylolytic bacteria, decreasing their population, hence reducing the rate and extent of ruminal starch fermentation (Hoover and Miller, 1991; Kotarski et al., 1992). This phenomenon has been extensively reviewed by Cerrilla and Martínez (2003). As a result, protozoa may influence the site of starch digestion, shifting starch digestion to the small intestine (Mendoza et al., 1993).

Ruminal fermentability of starch is highly variable, ranging from <30 to >90% of starch intake (Nocek and Tamminga, 1991; Larsen et al., 2009). In a recent meta-analysis, Moharrery et al. (2014) used *in vivo* starch digestion data from 66 publications and reported that the ruminal starch digestibility in dairy cows varied greatly from 224 to 942 g/kg. The intricate interrelations among several intrinsic and extrinsic factors such as grain/endosperm type, starch type, grain processing, conservation method, chemical alterations, ration composition (i.e. interactions with other diet ingredients) and animal factors (such as feed intake level, mastication, passage rate and absorption capacity, rumen microbial population and degree of adaptation of ruminal microbiota to the diet) affect both the site, rate and the magnitude of starch digestion, and therefore the profile and yield of VFA produced (Huntington et al., 2006; Reynolds, 2006; Giuberti et al., 2014).

The effects of these intrinsic and extrinsic factors on starch digestion in dairy cows have been extensively reviewed (Nocek and Tamminga, 1991; Huntington, 1997; Mills et al., 1999a; Theurer et al., 1999; Firkins et al., 2001; Huntington et al., 2006; Giuberti et al., 2014). Predominantly grain type and processing have been reported to profoundly affect both the rate and extent of starch digestion of cereal grains both in the rumen and post rumen (Firkins et al., 2001; Offner et al., 2003). The starch-protein matrix has been recognised as the physicochemical impediment to starch digestion (Philippeau et al., 2000), and responsible for differences in starch digestion among cereal grains (McAllister et al., 1993). For instance, starch in oats, wheat and barley are more readily degraded than the starch in maize and sorghum, with the latter being the least degradable (Theurer, 1986; Herrera-Saldana et al., 1990; Huntington, 1997; Firkins et al., 2001; Ferraretto et al., 2013), due to a dense protein matrix within the endosperm (McAllister et al., 1993). The starch-protein matrix encapsulates starch granules preventing microbial colonization and retards penetration by amylolytic enzymes. Moreover, vitreousness of the

endosperm, which increases with advancing maturity has been demonstrated to be negatively related to ruminal starch digestibility (Philippeau and Michalet-Doreau, 1997; Philippeau et al., 2000; Correa et al., 2002; Taylor and Allen, 2005; Ngonyamo-Majee et al., 2008; Lopes et al., 2009; Hoffman et al., 2010). In the vitreous endosperm, starch granules are densely packed within a protein matrix, and there is a greater concentration of prolamin protein (Philippeau et al., 2000; Larson and Hoffman, 2008), which is innately insoluble in rumen fluid, limiting the accessibility of hydrolytic enzymes to starch unlike floury endosperm (Kotarski et al., 1992). Depending on the grain processing method and the degree of processing, processing of cereal grains (grinding, rolling, steam rolling, steam flaking, etc.) increases ruminal starch (Huntington, 1997; Rowe et al., 1999; Theurer et al., 1999; Callison et al., 2001; Firkins et al., 2001; Rémond et al., 2004; Blasel et al., 2006; Ferraretto et al., 2013). This will result in greater net energy for lactation (NE_L) (Theurer et al., 1999; Firkins et al., 2001). Grain processing disrupts the pericarp and reduces the particle size, which increases the surface area for bacterial attachment and enzymatic degradation (Huntington, 1997). Depending on the moisture content and period, ensiling breaks down the starch-protein matrix by proteolytic activity, thereby increasing starch fermentability (Philippeau and Michalet-Doreau, 1998; Jurjanz and Monteils, 2005), (Hoffman et al., 2011; Ferraretto and Shaver, 2012; Ferraretto et al., 2015). Furthermore, increasing starch intake reduces starch digestion in the rumen (Nocek and Tamminga, 1991; Firkins et al., 2001; Patton et al., 2012; Moharrery et al., 2014), due to increased rumen passage rate (McCarthy et al., 1989), thereby reducing the time for starch hydrolysis in the rumen (Cerrilla and Martínez, 2003). Moharrery et al. (2014) reported a decrease of 14 g in starch digestibility for each kg increase in daily starch intake. Similarly, Patton et al. (2012) reported a decrease in rumen starch digestion from 75% at low starch intake to 60% when more than 4 kg of starch is consumed per day, increasing the amount of starch escaping the rumen to the small intestine.

2.5.2. Post-ruminal starch: digestion, end-products and limitations

Starch that escapes rumen fermentation can be enzymatically digested in the small intestine, to provide glucose that is absorbed or metabolized to lactate (Reynolds et al.,

2003), or escape enzymatic digestion to be fermented in the hindgut (caecum and colon) to yield volatile fatty acids (VFA) and microbial protein (Huntington et al., 2006). Otherwise, it is excreted in faeces (Figure 2.2). The digestion and absorption of starch in the small intestine occurs in three distinct phases (Owens et al., 1986; Huntington, 1997; Harmon et al., 2004; Huntington et al., 2006). The first phase of starch digestion in the small intestine begins in the lumen of the duodenum with the secretion and action of pancreatic α -amylase. The pancreatic α -amylase initiates the breakdown of the large starch molecules (amylose and amylopectin) at the α -(1,4) linkages producing maltose, maltotrioses, and α -limit dextrins. The second phase occurs at the brush border membrane, where these smaller polysaccharides (maltose, maltotrioses, and α -(1-6)-glucosides) are digested by enzymes found in the brush border, such as maltase and isomaltase, into glucose units. Finally, the released glucose is then transported out of the intestinal lumen and into the portal circulation (Huntington et al., 2006).

Similar to ruminal fermentability, small intestinal starch digestibility is highly variable, ranging from 40 to 85% of starch entering the duodenum (Owens et al., 1986; Huntington, 1997; Reynolds, 2006). A recent meta-analysis reported an average small intestine starch digestibility of 606 g/kg, ranging from 114 to 901 g/kg of starch entering the small intestine (Moharrery et al., 2014). Ruminal starch that escapes fermentation varies from 50 to 650 g/kg of starch ingested (Huntington, 1997). Numerous studies show that although the potential of lower intestinal starch digestion is considerable, the capacity is still limited (Reynolds, 2006). The overflowing of starch to the small intestine could overwhelm the capacity of starch digestion of the small intestine, resulting in reduced starch digestibility (Matthe et al., 2001; Oba and Allen, 2003b; Huntington et al., 2006; Reynolds, 2006; Ferraretto et al., 2013). Potential factors limiting small intestinal starch digestion, such as the adaptation of intestinal enzymes like pancreatic α -amylase (Huntington, 1997; Abramson et al., 2002, 2005; Richards et al., 2002; Huntington et al., 2006), brush-border carbohydrases (Kreikemeier et al., 1991; Kreikemeier and Harmon, 1995), and glucose transporters (Bauer et al., 2001; Harmon and Mcleod, 2001; Rodriguez et al., 2004) to increased postruminal starch supply have been questioned (Owens et al., 1986; Nocek and Tamminga, 1991; Mills et al., 1999b; Cerrilla and Martínez, 2003; Harmon et al., 2004; Huntington et al., 2006; Reynolds, 2006). The

adaptive response of intestinal enzymes and transporters to increased dietary starch remains a biological enigma (Huntington et al., 2006). In an effort to expose factors limiting starch digestion in the small intestine, other studies (Owens et al., 1986; Nocek and Tamminga, 1991; Theurer et al., 1999; Oba and Allen, 2003a; Taylor and Allen, 2005; Huntington et al., 2006) have suggested that intestinal starch digestion is not entirely limited by enzyme activities but also by factors that reduce the digestion of starch in the rumen such as grain particle size, endosperm type, passage rate, etc. Moharrery et al. (2014) indicated that the decreased starch digestion in the small intestine with increasing ruminal starch escape, reported in some studies, is confounded with the starch source as well.

Starch digested in the large intestine ranges from 44 to 46% of the starch the caecum (Huntington et al., 2006), corresponding to an average of 52.3 g/kg of starch intake (Moharrery et al., 2014). Digestion of starch in the large intestine is limited by relatively short residence time, and possibly because the starch particles, having resisted digestion in the rumen and small intestine, are inherently resistant to digestion (Deckardt et al., 2013). Although starch digestion in the large intestine is similar to that of the rumen, the large intestine is regarded as the least efficient site of starch digestion because only VFA are absorbed, while the resulting microbial protein gets excreted in the faeces together with undigested starch. Moreover, excessive flow of fermentable carbohydrates to the hindgut can lead to excessive fermentation in the hindgut which can result in hindgut acidosis (Gressley et al., 2011). Total tract digestibility of starch in dairy cows ranges therefore from 70 to 100%, with lower estimates mainly resulting from insufficient starch degradation in the rumen (Theurer et al., 1999; Firkins et al., 2001), as post rumen starch digestion does not completely compensate for ruminal starch escape (Ferraretto et al., 2013).

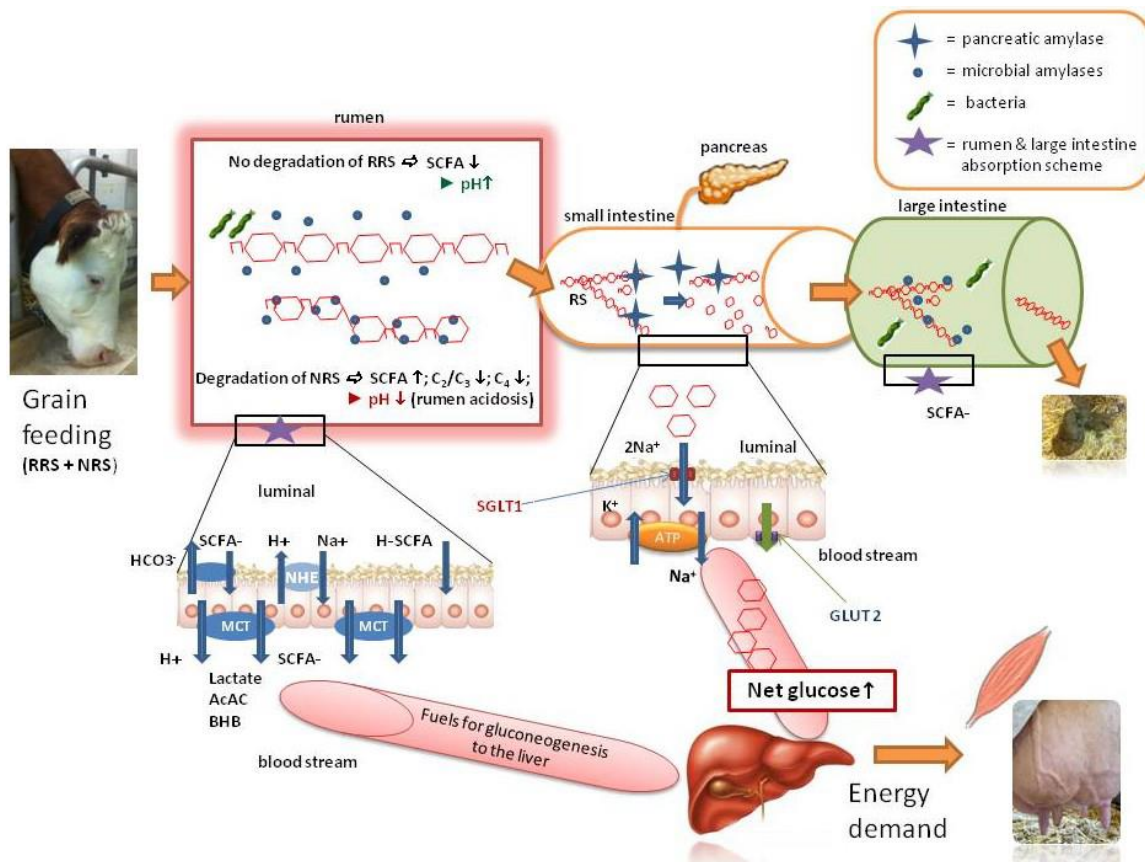


Figure 2. 2. Starch digestion and utilization in the dairy cows (from: Deckardt et al., 2013).

2.6 Effects of dietary starch on productivity of transition cows

Increased energy demands coupled with reduced DMI in transition cows causes a NEB, which promotes mobilization of body fat to meet the energy demand of high genetic merit dairy cows. Because DMI invariably drops prior to calving, a substantial amount of intense research has been conducted on nutritional management of transition cows over the past 20+ years (Grummer, 1995), focusing on increasing the energy density of diets during the transition period (Grummer, 1995; Rabelo et al., 2003; Overton and Waldron, 2004; Cardoso et al., 2013). Consequently, numerous nutritional strategies have been developed and deployed with the aim of attaining a successful transition by supporting metabolic adaptations and minimize the magnitude and duration of decreased DMI during this period. Subsequently, NEB and other complications such as metabolic disorders, impaired reproduction and production experienced during this period may be minimized.

2.6.1. Response to prepartum dietary starch content and sources

Although a substantial amount of research has been conducted to investigate the effects of increasing fermentable carbohydrates of the prepartum diet on DMI, energy balance and production performance during the transition period, the results are still inconclusive as reviewed previously (Grummer, 1995; Overton and Waldron, 2004; Remppis et al., 2011). Moreover, most studies were based on NFC content but not particularly on starch content and quality, resulting in the NFC content and energy content of the prepartum diets could have been confounded (Smith et al., 2008).

Nevertheless, taking the confounding effects of energy intake in previous studies into account, Smith et al. (2008; 2005) formulated iso-energetic diets that differed only in the carbohydrate sources of the total mixed ration (TMR), containing a high proportion of starch-based cereals (28% starch content) or based upon non-forage fibre sources (18% starch content). The pre and postpartum DMI was not affected by sources of carbohydrate in the prepartum diet (Smith et al., 2005). Contrary, a series of experiments, summarised by Overton and Waldron (2004), showed increased prepartum DMI as a result of greater ruminal DM digestibility allowing for faster rumen digesta turnover (Rabelo et al., 2003; Guo et al., 2007). DMI however decreased at a greater rate in the final week prior to calving (Minor et al., 1998; Rabelo et al., 2003). In contrast, Smith et al. (2005) found no differences in the pattern of the decreasing DMI before calving. According to Smith et al. (2005) a dramatic drop in DMI prior to calving reported by Rabelo et al. (2003) and Minor et al. (1998) could be a phenomenon associated with overall energy provided from carbohydrate sources. As reported in previous reviews (Ingvarsen and Andersen, 2000; Hayirli et al., 2002; Grummer et al., 2004) feeding high energy diets during the prepartum period is associated with a greater decline in DMI as parturition approaches. Furthermore, evidence is growing that overfeeding energy prepartum results in a slower increase in DMI and is detrimental to the cow health (Herd, 2000; Dann et al., 2006; Guo et al., 2007; Janovick and Drackley, 2010). Although, Grummer (1995) found a significant positive correlation between pre and postpartum DMI, which becomes stronger as the cow approaches parturition (Grummer et al., 2004), evidence that increased prepartum DMI increases postpartum DMI are limited. Most studies reported no effects on postpartum

DMI (Grum et al., 1996; Holcomb et al., 2001; Keady et al., 2001; Smith et al., 2005; Guo et al., 2007), with some studies reporting a very modest effect (Doepel et al., 2002; Rabelo et al., 2003).

Prepartum sources of carbohydrates or increased starch content did not affect milk yield (Overton and Waldron, 2004; Smith et al., 2005) and it has no carry over effects on the subsequent production performance (Rabelo et al., 2003). Doepel et al. (2002) suggested that the reliance on the mobilization of body fat to support milk synthesis may modulate the effect of prepartum treatment on milk yield. The effects of increasing starch or NFC content of the prepartum diet on milk fat content is inconsistent, with some studies reporting no effect (Mashek and Beede, 2000; Smith et al., 2005; Guo et al., 2007) and others reported increased (Keady et al., 2001) and decreased milk fat (Minor et al., 1998; Doepel et al., 2002). This inverse effect may be attributed to the level of plasma NEFA from the mobilisation of body fat reserves, which is positively correlated to milk fat content (Pullen et al., 1989). Keady et al. (2001) observed a greater mobilization of fat in early lactation cows fed concentrate supplementation in prepartum diets, hence high plasma NEFA and subsequently high milk fat content, contrary to Doepel et al. (2002) and Minor et al. (1998) who reported a reduced plasma NEFA, which may limit milk fat synthesis. It has been reported that circulating NEFA are utilized for approximately 40% for milk fat in the first days of lactation (Bell, 1995). Milk protein content is not affected by starch content of the prepartum diet in most studies (Keady et al., 2001; Doepel et al., 2002; Rabelo et al., 2003). Some studies however reported a positive response of milk protein with an increase of starch prepartum (Minor et al., 1998). A positive response could be attributed to increased microbial growth and protein synthesis in response to increased ruminally available carbohydrates (Oba and Allen, 2003b; Firkins et al., 2006).

2.6.2. Response to postpartum dietary starch content and sources

Although numerous studies have evaluated the carryover effects of prepartum diets on postpartum metabolism and performance, there is a paucity of studies on the whole transition phase in relation to starch intake and its effects on DMI and production. Most studies on transition cows have focused on the dry period and those done

postpartum did not start until three to four weeks after calving, when the period of extreme changes is over and cow variability is reduced. The lack of studies during the transition phase could be attributed to the delicacy of this stage, with the endocrine, metabolism, and physiology changes occurring, increasing cow's variation, and thereby making this stage more difficult to study and to interpret results. In addition, the short time frame of the transition period makes it difficult to gather enough animals with similar productivity characteristics to have enough statistical power. As a result, nutritional recommendations of fresh cows are based on field experience and limited research. Typically, fresh cow diets are formulated to support milk production, reduce adverse effects of NEB, hence mitigating the mobilization of body fat and accelerating the removal of hepatic NEFA, to safeguard the health of transition dairy cows and support the return to reproductive capacity.

Nevertheless, recently, few researchers have investigated the effects of high starch versus low starch content of diets fed to fresh cows on DMI, blood metabolites and lactation performance. The results of these studies have however been contradictory (Table 2.2), possibly due to the interactions among diet starch concentration and fermentability, diet forage NDF (fNDF) concentration and duration of treatments (Albornoz and Allen, 2018). Albornoz and Allen (2018) evaluated the effects of starch concentration (28% vs. 22%) for the first 23 days postpartum (PP) by substituting maize grain for soyhulls, keeping fNDF and the filling effect of diets constant. The latter authors reported that diet starch concentration had no effects on DMI, milk yield or milk components.

McCarthy et al. (2015a) hypothesized that feeding diets with greater propiogenic potential would increase milk yield because of increased gluconeogenic capacity (Drackley et al., 2001), and it would not have hypophagic effects on DMI because of the increased glucose demand after calving (Bell, 1995). This is due to the fact that following calving, glucose requirements of the lactating mammary gland is estimated to be more than double than that of gravid uterus prepartum (Bell, 1995). On the other hand, Allen and collaborators (2000, 2009) proposed that feeding diets that promote greater ruminal propionate production in early lactation would be hypophagic and further exacerbate the state of NEB due to the increased oxidation of propionate in the liver. Moreover, according to the H₂O₂ theory, elevated NEFA oxidation in the liver suppresses feed intake (Allen et al.,

2009). To study this hypothesis McCarthy et al. (2015a) fed cows either a high (26.2%) or a low starch (21.5%) fresh cow diet (from calving to 21 days). The experimental diets were based on ground maize partially replaced with citrus pulp and soy hulls in the low starch diet. Cows were fed the same diet prepartum (17.4% starch) and from 22 to 63 DIM (26.2% of starch). McCarthy et al. (2015a) reported that all cows had a similar overall DMI, expressed as kilograms per day, during the early lactation period, but cows fed a high starch diet (26.2%) had a faster increase in DMI and milk yield during the first 63 DIM than cows fed a low starch diet (21.5%). In addition, feeding a high starch diet increased glucose and insulin concentrations and decreased NEFA and BHBA concentrations postpartum, indicating a better energy status (McCarthy et al., 2015c).

In agreement, other studies (Andersen et al., 2002, 2003, Rabelo et al., 2003, 2005) had reported that feeding more propiogenic diets have positive effects on either DMI, milk yield and energy balance or both. These studies suggest that feeding more fermentable diets during early lactation does not decrease DMI or negatively impact performance or health of dairy cows. For instance, Rabelo et al. (2003, 2005) observed that cows fed the high NFC diet postpartum (47.2% NFC) from ground maize had a quicker increase in milk production postpartum, and tended to have higher DMI and a higher energy intake during the first 20 DIM, than cows fed a low NFC diet postpartum (41.1% NFC). Similarly, Andersen et al. (2003, 2002) observed that feeding a high starch diet (26.7% starch) based on rolled barley from calving to 8 weeks postpartum did not affect DMI and BCS change in early lactation, but it increased the net energy (NE) intake and milk yield in the first 8 weeks of lactation compared to cows fed a low starch diet (17.8% starch). Cows fed the high starch diet (26.7% starch) had a higher concentration of glucose and insulin, a lower BHBA concentration and a tendency toward lower NEFA concentration in week 2 and 7 after calving (Andersen et al., 2002). Moreover, Andersen et al. (2003) observed that feeding a high energy density diet decreased liver TAG content and increased LCFA oxidation capacity in early lactation. Similarly, Rabelo et al. (2005) observed a more favorable metabolic profile when cows were fed a high energy density diet, i.e. a lower BHBA concentration, a lower liver TG content, and higher plasma concentration of glucose and insulin. However, no effects on NEFA concentration were observed, indicating that a higher concentration of insulin failed to decrease the NEFA

concentration, suggesting that the antilipolytic effects of insulin did not happen (Rabelo et al., 2005).

Dyck et al. (2011) examined the effects of dietary starch sources and content in the immediate postpartum period on plasma metabolites and hormones and ovarian follicular development. Cows were fed diets containing 45% barley-based concentrate and 10% alfalfa hay, and either 45% alfalfa silage (24% starch), 45% barley silage (23% starch), or 41% barley silage plus 4% maize starch (27% starch) during the first 70 DIM (Dyck et al., 2011). Starch supplementation (23.3% vs. 26.7%) had no effect on DMI, BCS, blood metabolites and milk yield or milk fat or protein content (Dyck et al., 2011). Dyck et al. (2011) concluded that overall, dietary starch source and concentration had little effects on productivity and metabolic status of postpartum cows. Williams et al. (2015) studied the effects of starch content of the postpartum ration (21.3% vs. 27.2%) on subacute ruminal acidosis and the acute phase response in Holstein cows. Cows were fed a close-up diet of 15.5% starch, and ground maize was replaced with a mixture of soyabean hulls and wheat middlings. The DMI, NEFA and BHBA concentrations were not affected by diets, however, cows fed the high starch diet produced more milk than those fed the low starch diet (40.2 vs. 43.9 kg/d) (Williams et al., 2015). Higher starch levels contributed to lower rumen pH, increasing the risk of sub-acute ruminal acidosis, and inflammation during the fresh period (Williams et al., 2015). Interestingly, in contrast, a trial by Nelson et al. (2011) reported that cows transitioned from a prepartum diet (13.5% starch) to a high (25.5%) starch diet provided by maize meal had a decreased DMI and milk yield during the first 91 DIM compared to cows fed a low starch diet (21%). However, cows fed a high starch diet had lower NEFA and BHBA concentrations than cows fed a lower starch diet (Nelson et al., 2011). In the Nelson et al. (2011) study, maize meal was partially replaced with non-forage fibre sources (NFFS; soybean hulls and wheat middlings) in the low starch diet. Concurring with Nelson et al. (2011), in a recent study, Keskin et al. (2016) investigated the effects of different starch levels (24.5% vs. 16%) based on ground maize and barley on milk production, blood metabolites, and reproductive traits, and reported a lower DMI and milk production in cows fed a high starch diet, while BCS and blood metabolites did not differ.

The discrepancy could be attributed to the differences in the starch content and fermentability of the prepartum diet, starch sources and its processing and by the differences in NDF content or digestibility of diets or the diet forage NDF (fNDF) concentration, which may have affected the response to the starch levels fed postpartum. For instance in comparison with the study by Nelson et al. (2011), McCarthy et al. (2015a) fed 17.4% of starch based on maize during the close-up period, adapting the rumen and microbes to a high starch diet (Goff and Horst, 1997; Overton and Waldron, 2004) before the level of starch was increased to 26.2% vs. 21.5% in the postpartum diets, hence a better response. Moreover, the differences in the NDF concentration and digestibility during the fresh period may have affected the response to the starch content (McCarthy et al., 2015a; c). Researchers are speculating that there are interactions between starch and fibre levels in the postcalving diet. The interest is on the physically effective fibre (peNDF) which is known to contribute toward rumen mat formation and proper rumen function, and seems to be more important in fresh cow diets (McCarthy et al., 2015a; c). Moreover, forage NDF (fNDF) is known to be more filling than nonforage NDF (Allen, 2000).

An increase in milk yield in early lactation (Andersen et al., 2002; Rabelo et al., 2005; McCarthy et al., 2015a) is likely a result of greater gluconeogenic precursors supply to the liver that is provided by the higher-energy propiogenic diets (Maltz et al., 2013; McCarthy et al., 2015a). Generally, increased DMI postpartum results in lower circulating NEFA as a result of reduced lipid mobilization, and is associated with improved performance, health and less severe NEB (Ingvarsen and Andersen, 2000; Adewuyi et al., 2005). Moreover, ruminal propionate is a major glucogenic precursor for hepatic gluconeogenesis, accounting for 60-74% of the total hepatic glucose production (Reynolds et al., 2003; Aschenbach et al., 2010; Larsen and Kristensen, 2013), and a potent insulin secretagogue (Harmon, 1992; Grummer, 1995). Consequently, a higher concentration of blood glucose and insulin was observed in cows fed higher starch diets (Andersen et al., 2002; Rabelo et al., 2003, 2005; McCarthy et al., 2015a). The liver adapts to propionate supply during early lactation, by increasing its capacity to convert propionate to glucose to meet the increased demands of glucose for milk production (Drackley et al., 2001). This high gluconeogenic capacity does not exist either before

calving or at peak lactation (Drackley et al., 2001). Andersen et al. (2002) observed that cows fed a higher energy diet had a greater liver capacity to convert long chain fatty acids to CO₂, lower capacity to convert fatty acids to triglycerides in the liver, hence low liver TAG content, and lower blood ketones compared with cows fed a low energy diet. Feeding propiogenic diets increases liver glucose output, thus resulting in less dependence on adipose mobilization and, consequently, in better energy status.

The effects of a postpartum starch diet on early lactation milk components have not been consistent across studies. Andersen et al. (2003) observed that cows fed a high starch (26.7 vs. 17.8%) diet postpartum had a lower percentage of milk fat, higher milk protein and lactose content. Rabelo et al. (2003) reported a lower milk protein content in cows fed a higher energy diet postpartum from ground maize, although other milk component concentration and yield were unaffected by diet. McCarthy et al. (2015a) reported cows fed a high starch diet (26.2%) had lower milk component concentrations (milk fat, protein, lactose and total solids) than cows fed a low starch diet (21.5%). Milk composition is influenced by several factors such as breed, genetic variation within breeds, health, environment, management practices, stage of lactation, level of milk production and nutritional management. Generally, the high starch or NFC diets result in increased milk protein with associated low milk fat concentrations (Theurer et al., 1999; Firkins et al., 2001; Ferraretto et al., 2013). High starch intake results in increased ruminal microbial growth and protein synthesis (Theurer et al., 1999; Firkins et al., 2006; Jenkins and McGuire, 2006), greater propionate concentrations (Oba and Allen, 2003a; Firkins et al., 2006) and greater mammary uptake of amino acids (Huntington, 1997; Theurer et al., 1999; Rius et al., 2010), subsequently increasing milk protein concentration. In contrast, milk fat is predominantly triglycerides (>95%) (Jensen, 2002), which is derived from *de-novo* synthesis in the mammary epithelial cells (from acetate and β -hydroxybutyrate) and preformed fatty acid uptake from blood circulation originating from either the diet or mobilized body fat (Bauman and Griinari, 2003). Therefore, milk fat concentration is affected significantly by physiological factors mainly a change in energy balance and nutritional factors (Sutton, 1989; Grummer, 1991; Palmquist et al., 1993; Bauman and Griinari, 2001; Harvatine et al., 2009). According to Palmquist et al. (1993), lipogenesis in the mammary tissues is usually decreased by high intakes of cereal grains, decreasing

the availability of milk fat precursors (Bauman and Griinari, 2001) and consequently milk fat. There is a negative relationship between milk fat content and ruminal digestibility of starch (Firkins et al., 2001). Feeding excessive amounts of readily fermentable carbohydrates, such as high starch, decreases fibre digestion and rumen pH and thus decreases the production of acetate and butyrate in the rumen, which are the precursors for milk fat synthesis (Jenkins and McGuire, 2006). Alterations of the rumen environment and fermentation, mostly notably a decrease in rumen pH (Allen, 1997) and a decrease in the acetate to propionate molar ratio causes diet-induced milk fat depression (MFD) (Bauman and Griinari, 2001; Oba and Allen, 2003a; Bradford and Allen, 2004). Sutton (1989) estimated that up to 80% of the variation in milk fat content can be accounted for by changes in molar proportions of VFA in the rumen. Moreover, recent evidence indicates that MFD is caused by the shift in rumen biohydrogenation of unsaturated fatty acids and the passage of specific fatty acid intermediates such as trans-10, cis 12 conjugated linoleic acid (CLA) out of the rumen which, when absorbed, result in a decreased expression of lipogenic enzymes and a reduction in milk fat synthesis in the mammary gland (Bauman and Griinari, 2003; Shingfield and Griinari, 2007; Bauman et al., 2011). Feeding large quantities of maize increases the risk of MFD because of increased production of conjugated linoleic acid, inhibiting milk fat synthesis (Griinari et al., 1998; Bradford and Allen, 2004). Collectively, there are numerous theories that have been postulated and examined over the last century to explain the mechanism of reduced milk fat or MFD with increasing starch intake or glucose infusion/intake. Three prominent theories; glucogenic-insulin theory, trans fatty acid theory and biohydrogenation theory, have been debated as extensively reviewed (Bauman and Griinari, 2001; Jenkins and McGuire, 2006; Shingfield and Griinari, 2007; Harvatine et al., 2009; Lock, 2013; Tripathi, 2015).

2.6.3. Response to starch fermentability

Apart from altering starch concentration, starch fermentability of the diet is also of importance during the transition period as it affects total tract starch digestibility, ruminal pH and fibre digestibility, the type, amount, and temporal absorption of end products (e.g.

acetate, propionate, lactate, glucose) available to the cow and feed intake (Allen, 2000). The effect of ruminal fermentability of starch diets fed during both prepartum and immediate postpartum has been investigated by varying fermentability of the dietary starch by processing (Dann et al., 1999) or feeding different sources of starch (Santos et al., 1999; Alamouti et al., 2009; Sadri et al., 2009; Mikuła et al., 2011; Fatahnia et al., 2012; Rockwell and Allen, 2016; Albornoz and Allen, 2018).

The effects of starch fermentability on DMI, energy balance and production performance are not consistent in the literature. Albornoz and Allen (2018) reported that feeding a highly fermentable starch source (high-moisture maize) during the early PP period (first 23 DIM) decreased DMI and milk yield and milk components compared with a less fermentable starch source (dry ground maize). The depression in DMI was greater when fed in the higher fermentable starch diet. Conversely, Rockwell and Allen (2016) reported that high moisture maize increased milk yield more for the first 28 DIM compared to dry maize, however no effects on DMI were reported. Dann et al. (1999) observed that increasing ruminal starch fermentability by steam flaking maize (39% total NFC content of the diet) from 28 d prior to calving to 63 DIM increased milk yield during the first 63 DIM, and decreased prepartum and postpartum NEFA concentrations and milk fat content. Cows fed steam-flaked maize tended to increase prepartum DMI but tended to decrease DMI over the first 63 DIM compared to cows fed dry-cracked maize (Dann et al., 1999). Santos et al. (1999) fed cows diets containing grain as steam-flaked sorghum or steam-rolled maize (39% of the diet) from 5 to 50 DIM. The DMI, milk yield and composition were not affected by grain processing (Santos et al., 1999). However, Santos et al. (1999) observed that cows fed steam-flaked sorghum tended to have higher DMI than those fed steam-rolled maize during the first 45 DIM.

When ground barley grain was substituted with ground maize neither DMI (pre- or postpartum), BW, net energy balance nor milk yield and composition were affected (Sadri et al., 2009). Moreover, Mikuła et al. (2011) observed that the replacement of triticale grain (high rumen degradable starch) with maize grain (low rumen degradable starch) from 21 d before the expected calving date to 120 d of lactation did not affect DMI, milk yield or blood indices. However, the concentration of NEFA in the transition period was lowest in the maize grain fed cows, corresponding with the lowest body condition losses

(Mikuła et al., 2011). Garnsworthy et al. (2009) studied the effects of high rumen degradable starch (wheat grain) and high rumen bypass starch (flint maize grain) and reported no treatment effects on DMI, milk yield and energy balance. Cows were fed on a standard diet from the first 40 DIM and then fed on the treatment diets until 70 DIM. Energy balance was negative after calving in all groups, reaching a positive energy balance in week 7 or 8 postpartum (Garnsworthy et al., 2009). Fatahnia et al. (2012) also reported no differences in DMI and NEFA when cows were fed wheat or maize as the main source of starch in prepartum diets. However, feeding rolled wheat during the prepartum period lowered prepartum DMI, energy intake and balance as compared to ground maize, but increased DM and energy intakes postpartum (Alamouti et al., 2009). There was no effect of prepartum carbohydrate source on plasma glucose, NEFA, BHBA and insulin concentration during the transition period, but cows fed rolled wheat had less change in BCS and numerically lower NEFA postpartum than cows fed ground maize (Alamouti et al., 2009).

Some researchers suggest that the inconsistent effects of ruminally available starch sources on feed intake, milk yield and composition might be related to differences in the extent of starch fermentation in the rumen, origin and type of grain and processing method, level of intake, passage rate, sources of forage in the diet, forage to concentrate ratio and protein sources (Huntington, 1997; Dann et al., 1999).

Table 2. 2. Effects of increasing starch content of the transition diet on DMI, blood metabolites and milk yield and composition.

Reference	Dietary Treatment		Treatment period (number of cows)	Response to high starch diet		
	Starch source (%)	Starch content		DMI	Milk yield	Metabolic indices
Albornoz and Allen (2018)	Substituting corn grain for soyhulls	28 vs. 22	From calving to d 23 postpartum	No effects	No effects	Increased glucose and insulin
Andersen et al. (2003, 2002)	Whole crop barley silage and rolled barley Substituted for forage	26.7 vs. 17.8	-8 weeks before calving to 8 weeks postpartum (n=40)	No effects on DMI, tendency for 6% higher DMI Net energy intake increased by 28%	Increased by 18%	<ul style="list-style-type: none"> • Increased glucose and insulin concentrations. • Lower BHBA, a tendency toward lower NEFA • Reduced liver TAG by 48%
Rabelo et al. (2003, 2005)	Ground maize Substituted for forage	47.2% vs 41.1% NFC*	From calving to d 20 postpartum (n=60)	Higher DMI and energy intake in 30 DIM	Greater rates of increase in milk production	<ul style="list-style-type: none"> • Increased plasma glucose and insulin concentrations • Decreased BHBA concentrations d 7 and 21 postcalving • No effect on plasma NEFA • Lower liver TG
Dyck et al. (2011)	Barley silage and 4% supplemental starch (maize starch)	26.7 vs. 23.3	0 to 70 DIM (n=27)	No effect	No effect	<ul style="list-style-type: none"> • No effect

Nelson et al.(2011)	Kernel-processed maize silage and maize meal substituted with soybean hulls and wheat middlings	25.5 vs. 21.0	For the first 91 d postpartum (n=72)	Decreased	Decreased	
McCarthy et al. (2015a; c)	Brown midrib maize silage and maize dry meals substituted with citrus pulp and soybean hulls	26.2 vs. 21.5	During the first 21 d postpartum (n=70)	Increased faster	Faster increase in milk	<ul style="list-style-type: none"> • Lower NEFA and BHBA concentrations • Higher plasma glucose and insulin concentrations
Williams et al. (2015)	Ground maize substituted with soybean hulls and wheat middlings	27% vs. 21%	First 21 DIM (n=16)	No effect	Increased	<ul style="list-style-type: none"> • No effects on NEFA and BHBA • Lower rumen pH, increasing risk of rumen acidosis
Keskin et al. (2016)	Ground maize and ground barley	24.5 vs.16.3	0 to 80 DIM (n=23)	Decreased	Decreased	<ul style="list-style-type: none"> • No effects

*Starch values for diets were not reported.

DIM = days in milk.

2.7 Modulation of the site of starch digestion in transition cows

Controversy exists on the optimal site of starch digestion in dairy cows. It has long been known that starch digested and absorbed as glucose in the small intestine is more energetically efficient (25% more efficient) compared to the starch fermented in the rumen and absorbed as VFA to produce glucose from propionate (Huntington et al., 2006). The difference in efficiency is a result of losses of carbon as methane and CO₂ during rumen fermentation, other losses are due to microbial maintenance energy costs as well as to heat. However, Huntington et al. (2006) showed that an increased energetic efficiency of the small intestine is only possible when small intestinal digestibility exceeds 75%. When less starch is digested in the small intestine an increase in large intestine digestibility offsets this efficiency because only VFA are absorbed, whereas the resulting microbial protein gets excreted in the faeces together with indigestible starch (Harmon and Mcleod, 2001).

The effects of modulating the site of starch digestion has been studied in dairy cows through varying particle sizes and processing of grains (Callison et al., 2001; Rémond et al., 2004; Larsen et al., 2009), varying dietary starch intake, grain source or endosperm type (McCarthy et al., 1989; Philippeau et al., 1999; Taylor and Allen, 2005), and grain conservation method (Oba and Allen, 2003b; a). Some studies investigated the modulation of site of starch digestion by duodenal glucose infusion (Frobish and Davis, 1977; Lemosquet et al., 1997; Hurtaud et al., 1998; Rigout et al., 2002a; b, 2003) or abomasal starch infusion (Knowlton et al., 1998; Reynolds et al., 2001; Abramson et al., 2005; Larsen and Kristensen, 2009). In a summary of studies, Nocek and Tamminga (1991) concluded that there was no clear evidence that increased postruminal starch digestion increases milk yield or milk composition. Several studies suggest that more propionate to glucose enhances both protein efficiency (higher microbial protein yield and milk protein) and yield of milk (Theurer et al., 1999), while other studies show that glucose originating from ruminal escape starch can be used for milk synthesis (McCarthy et al., 1989). Moreover, other studies have shown that glucose from post-ruminal starch would be primarily used for body tissue protein and fat deposition rather than milk production

(Reynolds et al., 2001; Reynolds, 2006; Larsen and Kristensen, 2009). In a study, Knowlton et al. (1998) infused 1,500 g/d of hydrolyzed starch abomasally or ruminally in postpartum cows (4-12 weeks) and reported that not all available glucose is partitioned to the mammary gland in early lactating cows. The results have been equivocal. Besides, these studies were done outside the transition period, mostly after 60 DIM. However, according to Larsen et al. (2009) feeding rations that partly shift the site of digestion from the rumen to the small intestine is, in theory, advantageous to overcome some of the nutritional shortcomings associated with meeting nutrient needs of transition cows. Glucose infused into the abomasum is efficiently transferred to peripheral tissues in postpartum transition cows (Larsen and Kristensen, 2009, 2012), leading to an overall increased glucogenic status and decreased NEFA levels (Larsen and Kristensen, 2009). According to Larsen and Kristensen (2009), feeding diets that increase glucose absorption from the small intestine could be a safe and attractive strategy to obtain both increased direct glucose supply and decreased fat mobilization. Larsen and Kristensen (2012) reported that feeding ruminal escape starch (NaOH-treated wheat grain) induced the highest glucogenic status among the tested feeding strategies, which was obtained by a greater release of glucose from portal-drained viscera, splanchnic tissues, and the lowest dependency on Cori cycling of glucogenic carbon via lactate. Recently, Zou et al. (2015) concluded that starch infusion (800 g/d) did not enhance energy supplies to the mammary gland or improved the lactating performance in low yielding cows. Mikuła et al. (2011) replaced triticale (high rumen degradable starch) with maize grain (low rumen degradable starch) during the transition period and lactation. Although there was no significant impact of treatment on milk production and in most of the blood indices, the concentration of NEFA was lower for maize grain fed cows, corresponding to lower body condition losses during the transition period, indicating a reduced mobilization of fat reserves. Therefore, Mikuła et al. (2011) suggested that energy efficiency of maize starch entering the small intestine is higher in comparison to ruminally degraded starch. Moreover, Mikuła et al. (2011) suggested that maize grain in the transition period and lactation might be a more effective energy source for dairy cows than triticale grain. These contrasting results might be partially justified by the hepatic oxidation theory (Allen et al., 2009) and need further investigation to confirm if glucose from small intestine digestion

would be used for production needs in animals having a high production demand for energy, such as early lactation dairy cows.

2.8 The optimal starch strategy for transition cows

Different nutritional strategies of transition cows have been proposed, with the aim of minimizing the negative effects associated with the transition period such as decreased DMI, NEB, metabolic disorders and reduced production and reproduction performance. Over the past decades considerable research has focused almost exclusively on dry cows, resulting on nutritional strategies focusing on promoting the development of ruminal papillae from feeding supplemental starch-rich concentrates (Dirksen et al., 1985), which was not supported by applicable studies (Andersen et al., 1999; Reynolds et al., 2004) who observed minimal changes on papillae during the transition period. It is, however, supported that prepartum diets should promote ruminal microbial adaptation and provide increased amounts of propionate to support hepatic gluconeogenesis and microbial protein (Overton and Waldron, 2004).

Most recent emerging carbohydrate strategies of transition cows are mainly based on starch concentration and fermentability, fuelled by the hepatic oxidation theory for regulation of feed intake. According to the HOT, feed intake during the transition period is controlled primarily by oxidation in the liver, whereas ruminal fill becomes a primary limitation to intake as lactation progresses (Allen et al., 2009; Allen and Piantoni, 2013). Unfortunately, there are relatively limited studies conducted on how best to feed starch to transition dairy cows for optimal production, health and reproduction. The volatility that characterizes this period makes it difficult to conduct research (Drackley, 1999). Available studies on the effects of diet starch concentration and fermentability fed during the transition period on the DMI, production and metabolism have been equivocal as aforementioned. Nevertheless, recent ration strategies from limited research and field experiences are formulated based on the fact that cows vary in their response to diets through their lactation as their physiological state changes (Allen, 2000; Allen et al., 2009). Although there is no “one size fits all” it is recommended that the fresh cow (from calving to 21 d postpartum) diets should avoid inclusion of highly fermentable starch

sources, and provide adequate physically effective NDF (straw, hay) to maximize DMI and minimize ruminal acidosis and displaced abomasum. The starch content should not exceed ~25%, taking into account the amount fed on the prepartum diet and the high group diet, after the transition period. It is suggested that the starch level in the fresh cow diet should not be more than 8 to 10% higher than the prepartum diet. Overall, a fresh cow diet should be formulated considering the concentration of the dry and high group diets.

For cows in early lactation, after the fresh period as lactation proceeds and when serum NEFA and BHBA are lower, the diet should then contain highly digestible carbohydrates to maximize DMI and milk production (Dann and Nelson, 2011). The starch concentration of the ration should range from 25-30% (DM basis) depending on the forage, on the physically effective NDF content and the starch fermentability. It is advisable to group fresh cows separately and be fed a fresh cow diet.

2.9 Conclusion

Although nutritional strategies for transition dairy cows have evolved and refined over the past 2-3 decades based on both scientific research and field observations, the transition period remains problematic, with negative impacts on health, productivity and profitability of dairy farms. In recent years, there has been a surge of research interest in the prepartum and postpartum diets of dairy cows, motivated by discussions related to carbohydrate formulation of these transition diets and potential interactions with DMI, coupled with perpetual challenges of the transition cows. Although the results from limited experiments summarized in this review are not consistent, recent studies are starting to doubt and question the hepatic oxidation theory. Most of the recent research provide no evidence of negative effects of increased dietary starch content and starch fermentability on feed intake, in fact milk production increases. Further research is needed in this area to better understand the interaction between starch content and fermentability during the transition period and reduction in DMI for best nutritional strategies to attenuate the abrupt changes in nutrient supply, maximize DMI and reduce adverse effects of NEB, hence profitability of the dairy farms.

2.10 References

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CHAPTER 3

***In vitro* starch degradability and rate of starch degradation of different particle sizes of cereal grains commonly fed to dairy cows**

3.1 Abstract

Cereal grains in South Africa are traditionally milled before inclusion in the diet of dairy cows to enhance rate and extent of starch digestibility. When milled, even if using a single sieve, different grains result in different mean particle sizes and distributions, which may influence digestibility. The effects of milled grain particle size fractions on chemical composition, starch degradability and rate of starch degradation were examined. Samples of maize (1 and 2), sorghum, barley and wheat were ground through a 1- and 2-mm sieves, and then fractionated by sieving to obtain the following sizes: <250 (very fine), 250-500 (fine), 500-1180 (medium) and 1180-2000 μm (coarse). Fractions and unsieved samples milled using a 1-mm sieve were separately analysed for chemical composition and fermented *in vitro* using rumen fluid for 0, 3, 6, 9, 12 and 24 h to determine the starch degradability (S_d) and rate of starch degradation (k_d). Cereal grains varied in chemical composition, with the highest starch content observed in maize-1 (77.30%) and the lowest starch content in barley (63.33%). Particle size affected ($P < 0.0001$) the chemical composition of all grains, with generally highest starch in the smallest particles and highest NDF in the largest particles. Decreasing particle size increased S_d and k_d for all grains, with a larger effect on sorghum (24% vs. 52% S_d). The S_d (k_d), when pooling time points, increased from 37.39 (11.04) to 60.92% (23.33% h^{-1}) for maize-1, from 46.88 (18.46) to 63.69% (28.00% h^{-1}) for maize-2, from 24.46 (7.65) to 52.36% (13.84% h^{-1}) for sorghum, from 40.91 (14.10) to 58.38% (21.68% h^{-1}) for barley and 56.25 (15.28) to 71.49% (25.28% h^{-1}) for wheat. The effect of particle size was observed at all incubation times, with differences more evident between finer and coarser particles. Nutritional composition, starch degradability and rate of starch degradation vary across particle size, which could be useful in the formulation of rations to better satisfy the requirements of dairy cows in different physiological stages. This study suggests that starch digestion

could be shifted post-rumen based on particle size by reducing the amount fermented in the rumen, assuming starch will be digested post-uminally.

3.2 Introduction

Cereal grains are commonly used as a primary source of energy for dairy cows all over the world because of their high starch content, ranging from 57% in barley, up to 77% on dry matter (DM) basis, in wheat and maize (Herrera-Saldana et al., 1990; Huntington, 1997). High yielding dairy cows are fed diets containing high amounts of starch (25 to 35% of DM), mostly supplied by cereal grains to meet their energy demands for milk production (Giuberti et al., 2014). Maize followed by barley, wheat and sorghum grain are the most commonly used starch sources in South Africa for ruminant diets. Because of variable climates, growing conditions, geographic locations and genetic factors, these grains vary in terms of starch content and ruminal starch availability.

Starch in cereal grains is highly digestible (70-100% in dairy cows), when considering the total gastro-intestinal tract. However, the rate and extent of starch degradation in the rumen, which is the major site of starch digestion (Theurer, 1986), vary considerably (<30 to >90% of starch intake) among cereal grain sources and grain variety (Firkins et al., 2001; Offner and Sauvant, 2004; Larsen et al., 2009; Ferraretto et al., 2013; Moharrery et al., 2014). Predominantly, variation in the inherent physical and chemical structure of cereal grains (Kotarski et al., 1992; McAllister et al., 1993; Philippeau et al., 2000) and processing of grains (Huntington, 1997; Theurer et al., 1999; Firkins et al., 2001; Ferraretto et al., 2013) determine the ruminal starch availability and degradability. Consequently, these factors affect the profile and yield of volatile fatty acids (VFA) produced and microbial protein supply (Theurer et al., 1999; Huntington et al., 2006; Reynolds, 2006) and ultimately feed efficiency and performance of dairy cows. The rate and extent of starch digestion have been a subject of interest particularly in dairy cows, resulting in numerous coherent reviews and starch digestion models (Nocek and Tamminga, 1991; Huntington, 1997; Theurer et al., 1999; Mills et al., 1999; Firkins et al., 2001; Offner and Sauvant, 2004; Reynolds, 2006; Huntington et al., 2006; Patton et al., 2012; Ferraretto et al., 2013; Giuberti et al., 2014; Moharrery et al., 2014).

Cereal grains are typically processed, mainly by milling prior to feeding to maximize the utilization of starch by enhancing both the rate and extent of starch digestibility. Milling reduces the particle size, exposing the endosperm and increasing the surface area for bacterial attachment and enzymatic degradation, which can result in increased ruminal starch digestibility and net energy for lactation (NE_L; Huntington, 1997; Theurer et al., 1999; Firkins et al., 2001). Feed mills often use a single sieve when milling grains to a standard theoretical size. In South Africa, most feed companies use 3- or 5-mm sieve sizes for grains. However, depending on the type and operation of the mills, different grains and cultivars may result in different particle size distributions and geometric mean particle sizes (Rémond et al., 2004; Al-Rabadi et al., 2012; Dias Junior et al., 2016), which can affect starch digestibility (Huntington, 1997; Firkins et al., 2001). Similarly, *in vitro* digestibility procedures require samples to be ground to the same theoretical size, achieved mainly by passing through a 1mm sieve, producing a range of particle sizes which may influence starch digestibility.

Although the effects of processing and particle size of cereal grains on starch digestibility have been reported in previous studies (Al-Rabadi et al., 2009, 2012; Hoffman et al., 2012; Ferraretto et al., 2013; Gallo et al., 2016), studies investigating the effects of milled grain particle size fractions obtained by sieving on chemical composition and *in vitro* ruminal starch digestibility are limited. In most of the previous studies, grains were ground using two or more screens of different sizes or using different mills equipped with different screens to obtain ground samples for analysis. The objective of this study was to evaluate the effects of different particle size fractions on chemical composition and *in vitro* ruminal starch degradability of cereal grains commonly used in dairy cow diets. We hypothesized that, once ground cereal grains are fractionated by sieving, each fraction will be unique with regards to the chemical composition, starch digestibility and rate of starch digestion. This study may provide valuable insights on ruminal starch degradation that could enable us to better characterize grains and ultimately fine tune diets. Precision feeding starch using this approach may in fact maximize utilization of starch both in the rumen and postruminally to better meet the nutrient requirements of dairy cows in different physiological stages, maximizing dairy cow performance and rumen health.

3.3 Materials and Methods

3.3.1 Cereal grains, fractions and particle size preparation

Common cereal grains fed to dairy cows in South Africa (two maize batches and one batch each of sorghum, barley and wheat) were investigated. Cereal grains were obtained from a local supplier (Agricol, Cape Town, South Africa) and were separately ground to pass through a 1-mm sieve using a Wiley mill (Model 4, Thomas-Scientific, Swedesboro, NJ, USA). Ground grains were fractionated using a laboratory sieve shaker (Kingtest laboratory test sieve, Retsch GmbH, Series AS 200 basic, Germany) and four sieves, with mesh sizes of 2000, 1180, 500 and 250 μm , followed by a pan to recover particles less than 250 μm . To obtain larger particle sizes (1.18-2.0 mm), cereals were ground at 4.5 mm using a hammer mill (Scientec RSA Hammer mill Ser Nr 372; Centrotec) before sieving. Fractions were categorised as very fine (<250 μm), fine (250-500 μm), medium (500-1180 μm) and coarse (1180-2000 μm). Unsieved samples ground to pass through a 1-mm sieve were also analysed.

3.3.2 Chemical analysis and *in vitro* starch degradability

All fractions described above were subjected to chemical analysis and *in vitro* starch degradability. The dry matter (DM) and ash contents were determined according to the AOAC, (2002), official method 934.01 and 942.05, respectively. Crude protein was determined by the Dumas combustion method according to the AOAC, official method 992.15 using a LECO nitrogen analyser (model FP-528, Leco Corporation, St. Joseph, MI, USA). Neutral detergent fibre (NDF) was analysed, as described by Mertens (2002) using sodium sulphite and thermostable α -amylase, and the acid detergent fibre (ADF) according to AOAC (2002) method. The starch content was determined as described by Hall (2009). Briefly, sample of approximately 0.25 g was weighed into a 50 ml plastic test tube prior to adding 30 ml of 0.1M sodium acetate buffer solution (pH 5) containing 300 μL of thermostable α -amylase (Ankom; α -amylase, heat stable) to each test tube. Samples were incubated at 100°C in a water bath for one hour to gelatinise and partially hydrolyse starch. Samples were then cooled to 50°C before adding 300 μL of

amyloglucosidase, and incubated at 50°C for 2 h to completely hydrolyse starch into glucose. The entire sample content was then filtered through glass wool into a 100-ml volumetric flask and brought to volume with distilled water. Volumetric flasks were shaken gently to mix the content well, before 1 mL aliquots were transferred to Eppendorf tubes (Lasec, South Africa) and centrifuged for 10 min at 1000 x g. For absorbance, 300 µL of the GOPOD (Megazyme, Wicklow, Ireland) reagent solution was added to a microplate. A glucose standard curve was prepared using concentrations of 0, 2, 4, 6, 8, 10 µL of D-glucose standard solution (GOPOD, Megazyme, Wicklow, Ireland), diluted with distilled water. The 10 µL aliquots were then added to the microplate with the GOPOD reagent solution, incubated at 50°C for 20 min and absorbance was measured immediately (at least within 20 minutes) in a spectrophotometer (Spectrostar Nano, BMG Labtech) at a wavelength of 505 nm. The standard curve was used to determine the concentration of glucose, which was used to calculate the starch content of samples using a factor of 0.9. For fermented samples, the starch concentration was determined in the total content of the Erlenmeyer flasks (i.e. residue + medium). Soluble starch was therefore included in the resulting starch content. All chemical analyses were done in duplicate.

In vitro rumen degradability was measured according to the method of Goering & Van Soest. (1970), with slight modifications. Approximately 0.25 g of each sample was weighed into 125-ml Erlenmeyer flasks before the addition of 40 ml of *in vitro* buffer prepared according to Goering & Van Soest (1970). The buffer contained a macro-mineral solution containing (per L) 5.7 g Na₂HPO₄ anhydrous, 6.2 g KH₂PO₄ anhydrous, 0.6 g MgSO₄·7H₂O; buffer solution containing (per L) 4 g ammonium bicarbonate (NH₄HCO₃), 35 g sodium bicarbonate (NaHCO₃); trypticase; micro-mineral solution containing (per 100 ml) 13.2 g CaCl₂·2H₂O, 10 g MnCl₂·4H₂O, 1 g CoCl₂·6H₂O, 8 g FeCl₃·6H₂O; and resazurin (0.1% w/v solution); plus potassium hydroxide pellets (KOH), cysteine-HCL and sodium sulphide nonahydrate. Erlenmeyer flasks were placed in the water bath preheated to 39.5°C, closed with rubber stoppers and gassed with CO₂ to reduce the media pending incubation with rumen fluid.

Rumen fluid was collected from two ruminally cannulated, lactating Holstein cows in the morning (~07:00) before feeding, at the Welgevallen Experimental Farm of the Stellenbosch University. The cows were fed a total mixed ration (TMR) twice a day (07:30

and 17:00) formulated to meet their maintenance and production requirements. The TMR contained approximately 28% lucerne, 13% straw and molasses and 58% of a concentrate mixture fed twice a day, at 07:30 and at 17:00. The same cows were used during the entire experiment. Rumen fluid was collected directly from the ventral area of the rumen by hand using a cup, transferred to a pre-warmed thermos flask until filled, and then it was transported to the laboratory until used (within approximately 20 min). In the laboratory, the rumen fluid was filtered through two layers of cheese cloth, glass wool, and a double layer of 200 μ m mesh placed in a funnel into a 1-L pre-warmed Erlenmeyer flask. It was then flushed with CO₂ to purge out air before injecting 10 ml in each flask using a pre-warmed automatic syringe (Dosys™, Socorex, Switzerland). The medium in each flask was fully reduced, using resazurin as indicator, before injecting the rumen fluid. An anaerobic condition was maintained throughout the incubation period by continuous gassing with CO₂ using a pressurized manifold connected to each flask. Flasks were checked for leaks, and gently swirled from time to time during incubation using the shaker. Incubation times of 3, 6, 9, 12 and 24 h were used to determine the rate of starch degradation. After each incubation time, the respective flasks were removed from the incubator, flushed with cold distilled water immediately and placed in an ice bath to stop microbial activity. The residual starch was determined using the entire incubated content of samples in Erlenmeyer flasks (i.e. residue + medium) and therefore soluble starch was included in the resulting starch content. Three separate runs were conducted for each cereal fraction, in duplicates of each sample for each incubation time.

The protocol for rumen fluid collection used in this experiment was approved by the Research Ethics Committee for Animal Care and Use of Stellenbosch University (protocol number SU-ACUD14-00052), South Africa.

3.3.3 Calculations and models

The *in vitro* starch degradability was calculated as the ratio between the amounts of starch which disappeared at 3, 6, 9, 12 and 24 h of incubation and the amount of starch in the sample before incubation. Rates of starch degradation were then computed using a first order decay model, according to the following equation:

$$S(t) = S_{(0)} e^{-k_d(t-L)} + uS;$$

where $S_{(0)}$ is the size of the digestible soluble and insoluble starch at time 0; k_d is the fractional rate of starch digestion, L is the lag and uS is the estimated indigestible starch. Simultaneous estimations of the parameters k_d and L were initially obtained using PROC NLIN of SAS (version 9.3; SAS Institute, Inc., Cary, NC) and the Marquardt algorithm. The Marquardt algorithm was selected to improve the efficiency of providing least-squares estimation for the non-linear curve fitting approach. Non-linear regression was chosen as the standard procedure because the method assumes equal error at each observation and by simultaneously fitting all parameters to the data, the result provides the smallest residual sums of squared deviations. The necessity of establishing initial parameter values for the non-linear estimations was solved using a linear approach to seed the non-linear estimation as done by Grant and Mertens (1992). The log-linear approach of Van Soest et al. (2005) was used to generate the initial values for each sample to parameterize the decay model. The potentially digestible starch (pdSd) was calculated as starch – indigestible starch. The indigestible starch was then calculated using the pdSd, assuming 100% starch availability.

3.3.4 Statistical analyses

Data of the chemical compositions of the different grains and fractions were analysed according to a randomized complete block design using the GLIMMIX procedure of SAS (version 9.3; SAS Institute, Inc., Cary, NC) with a factorial arrangement of cereal grains, particle size and their interaction. The *in vitro* starch degradability values and the rates estimated by the nonlinear regressions were analysed as response variables by the GLIMMIX procedure of SAS (version 9.3; SAS Institute, Inc., Cary, NC) using a factorial arrangement of particle size, grain, time (for Sd only) and respective interactions. Fermentation run ($n = 3$) was considered a random effect. Differences between means were declared significant at $P \leq 0.05$ using LSMEANS and the Tukey adjustment. Trends were considered for statistical differences between $0.05 < P \leq 0.10$. Treatment results are reported as LSMEANS and respective standard errors of the mean (SEM).

3.4 Results and Discussion

3.4.1 Chemical composition of cereal grains and effects of varying particle sizes

Table 3.1 shows the chemical composition of the cereal grains used in the current study. The grains (maize-1, maize-2, sorghum, barley, and wheat) varied considerably in chemical composition, with the highest starch content observed in maize-1 (77.30%) and the lowest starch content in barley (63.33%). These results are in agreement with previous studies reporting the lowest starch content in barley in comparison to other cereals (Herrera-Saldana et al., 1990; Huntington, 1997; Lanzas et al., 2007). Depending on genetic factors, growing conditions and geographic location, starch content ranges from 72-78% for maize, 68-78% for sorghum, 67-77% for wheat and 57-74% (on DM basis) for barley (Herrera-Saldana et al., 1990; Huntington, 1997). Barley and sorghum resulted in the highest (27.10%) and lowest (10.92%) NDF content, respectively. In comparison to maize, wheat and sorghum, barley is known to contain a higher NDF content, ranging from 16 to 27% (Zhao et al., 2016), due to its fibrous hull, which comprises about 13% of its grain weight on average, and can range from 7 to 25% (Evers et al., 1999). This may also explain the highest ash content of barley (1.92%), when compared to the other cereals. In agreement with previous studies by Herrera-Saldana et al. (1990) and Lanzas et al. (2007), wheat showed the highest CP content (13.35%) and maize the lowest CP; 7.19 and 7.85% in maize-2 and maize-1, respectively. Compared to other cereals, wheat is known to have a higher CP content, ranging from 10-18.8% (McAllister and Sultana, 2011), with the maize CP content ranging from 7.5-11.5%.

Besides differences among cereal grains, variations in the chemical composition also exist within the same cereal types as observed between maize-1 and maize-2, possibly due to different genetic factors, agronomic practices, and growing conditions such as geographical and environmental variations and their interactions (Huntington, 1997; Dehghan-banadaky et al., 2007). Unfortunately, information on the two batches of yellow maize used in the study was not available. Overall, the chemical composition of cereal grains reported in this study is within the range of values reported earlier (Herrera-

Saldana et al., 1990; Huntington, 1997; Khorasani et al., 2000; Yang et al., 2000; Beauchemin et al., 2001; Stevnebø et al., 2009; McAllister and Sultana, 2011; Benninghoff et al., 2015; Zhao et al., 2016).

Table 3. 1. Chemical composition of the cereal grains used in the study (% of DM).

Component*	Cereal grains					SEM
	Maize-1	Maize-2	Sorghum	Barley	Wheat	
DM	86.86	86.57	87.62	87.71	87.75	0.09
Ash	1.14	1.15	1.15	1.92	1.54	0.03
Starch	77.30	75.84	76.86	63.33	68.01	0.68
CP	7.85	7.19	9.51	10.03	13.35	0.04
NDF	11.96	15.65	10.92	27.10	13.87	0.24
ADF	3.47	4.47	5.03	5.45	3.96	0.09

^{a-e} values within a row with different superscript differ ($P < 0.0001$). *DM= dry matter; CP= crude protein; NDF= Neutral detergent fibre; ADF= Acid detergent fibre.

Particle size had a significant effect ($P < 0.0001$) on the chemical composition of all grains, where the highest starch content was generally observed in the smallest particles and highest NDF and ADF in the largest particles (Table 3.2). Variation in the chemical composition among different particle size fractions within a grain are related to separation of components (i.e. pericarp, germ, or endosperm) of the grains. This is because nutrients are not equally distributed throughout the grains. For instance, maize endosperm contains a lower content of NDF (<4%) as compared to germ and pericarp (17% and 33%, respectively) (Van Kempen et al., 2003). When grains are milled, their components break and separate differently, hence their concentration in certain particle sizes once fractionated (Galyean et al., 1981). Previous studies have reported that most of the starch in the cereal grain is found in the flourey endosperm (Galyean et al., 1981), which would explain the higher starch content in the very fine particle size reported in this study. The highest content of NDF and ADF in the largest particle size (coarse) is attributed to the presence of the majority of the pericarp, which contains most of the fibre (~33% NDF in maize) (Giuberti et al., 2014). In all grains, as particle size decreased, starch content increased whereas the NDF content decreased.

Table 3. 2. Chemical composition of cereal grains varying in particle size and unsieved milled cereal grains in % of DM.

	Particle size						
	Very fine	Fine	Medium	Coarse	Unsieved	SEM	P-value
Maize-1							
DM	88.20	87.77	87.47	87.75	86.86	0.253	0.2443
Ash	1.48 ^a	1.10 ^b	1.12 ^b	1.40 ^a	1.14 ^b	0.003	0.0044
Starch	76.92 ^a	75.31 ^a	69.93 ^{ab}	65.06 ^b	77.30 ^a	4.250	0.0073
CP	7.04 ^b	8.04 ^a	7.82 ^a	7.75 ^a	7.85 ^a	0.024	0.0089
NDF	6.57 ^e	10.06 ^d	15.64 ^b	18.73 ^a	11.96 ^c	0.117	<0.0001
ADF	2.45 ^c	3.26 ^{bc}	4.36 ^b	7.36 ^a	3.47 ^{bc}	0.166	0.0004
Maize-2							
DM	85.50 ^{bc}	85.17 ^c	85.20 ^c	85.92 ^b	86.57 ^a	0.100	0.0008
Ash	1.24 ^b	1.07 ^b	0.80 ^b	1.85 ^a	1.15 ^b	0.015	0.0030
Starch	86.57 ^a	81.40 ^{ab}	80.81 ^{ab}	69.89 ^c	75.84 ^{bc}	3.442	0.0020
CP	6.66 ^c	7.91 ^a	7.60 ^{ab}	7.72 ^a	7.19 ^b	0.017	0.0012
NDF	5.04 ^d	12.23 ^c	17.03 ^b	27.70 ^a	15.65 ^b	0.120	<0.0001
ADF	1.79 ^c	3.93 ^b	4.54 ^b	8.77 ^a	4.47 ^b	0.120	<0.0001
Sorghum							
DM	87.13 ^{abc}	86.41 ^c	86.69 ^{bc}	87.43 ^{bc}	87.62 ^{ab}	0.049	0.0125
Ash	1.48 ^a	0.78 ^b	1.29 ^a	1.25 ^a	1.15 ^{ab}	0.011	0.0106
Starch	79.02	76.94	75.10	73.66	76.86	6.479	0.3889
CP	5.82 ^d	8.19 ^b	8.41 ^b	7.66 ^c	9.51 ^a	0.007	<0.0001
NDF	5.50 ^d	7.15 ^c	12.31 ^a	10.42 ^b	10.92 ^{ab}	0.152	<0.0001
ADF	2.38 ^d	4.08 ^c	5.79 ^b	7.34 ^a	5.03 ^b	0.036	<0.0001
Barley							
DM	88.40 ^a	87.50 ^{bc}	87.25 ^c	87.47 ^{bc}	87.71 ^b	0.010	0.0006
Ash	1.76 ^{ab}	1.68 ^b	2.03 ^a	1.65 ^b	1.92 ^{ab}	0.006	0.0148
Starch	65.35	63.45	57.86	67.17	63.33	6.507	0.0900
CP	9.03 ^c	10.63 ^a	10.94 ^a	10.63 ^a	10.03 ^b	0.012	<0.0001
NDF	10.73 ^c	16.97 ^b	29.40 ^a	30.25 ^a	27.10 ^a	2.165	0.0001
ADF	3.24 ^b	3.42 ^b	6.15 ^a	5.36 ^a	5.45 ^a	0.076	0.0004
Wheat							
DM	88.17	87.75	87.66	87.85	87.75	0.080	0.5001
Ash	1.30	1.13	1.50	1.34	1.54	0.015	0.0936
Starch	64.79	69.87	63.42	63.80	68.01	2.681	0.0383
CP	13.97 ^a	12.91 ^b	13.41 ^{ab}	13.10 ^b	13.35 ^{ab}	0.036	0.0169
NDF	11.82 ^b	12.79 ^b	14.44 ^{ab}	16.63 ^a	13.87 ^b	0.465	0.0060
ADF	3.37 ^{ab}	2.71 ^b	3.21 ^{ab}	4.06 ^a	3.96 ^a	0.068	0.0163

^{a-d} values within a row with different superscript differ ($P < 0.05$).

*DM= dry matter; CP= crude protein; NDF= Neutral detergent fibre; ADF= Acid detergent fibre

Moreover, our results showed that particle size fractions with a higher starch content had a lower protein content. According to Galyean et al. (1981), floury

endosperms are higher in starch content but lower in protein content as compared to vitreous endosperm and germ, which could explain the lower CP in fractions with higher starch content observed in this study. Dry matter content showed little variation between particle sizes for all grains. In case of unsieved ground grains (i.e. samples of grains milled to pass through a 1-mm screen but not sieved after milling), the differences observed in the chemical composition as compared to various particle sizes varied as shown in Table 3.2.

3.4.2 *In vitro* starch degradability and rate of starch degradation of cereal grains

Figure 3.1 shows the *in vitro* starch degradability and the potentially digestible starch (pdSd) of the cereal grains. The *in vitro* starch degradation resulted in significant differences in starch degradability ($P < 0.0001$) and rate of starch degradation ($P < 0.0001$) among cereal grains. When pooling time points, wheat resulted in the highest degradability (60.71%) followed by maize-2 (51.75%) and maize-1 (50.85%); barley (47.55%) and then sorghum (38.86%). The degradation rates had a different pattern, mainly because of the different estimated resistant starch (uS) and lag across the samples (Figure 3.2). In fact, the rate of starch degradation was highest in maize-2 (23.26% h^{-1}) followed by barley (20.58% h^{-1}), wheat (19.08% h^{-1}) and maize-1 (18.42% h^{-1}) and then sorghum (12.33% h^{-1}). As expected, wheat had the most degraded starch compared to the other cereals (Figure 3.1). The starch granules of wheat are loosely associated with the protein matrix and readily degraded by microbes, making it highly fermentable (McAllister and Cheng, 1996). The lower degradability of starch in barley (47.55%) relative to maize (50.85 and 51.75%, maize-1 and maize-2, respectively) was unexpected given that the protein matrix in barley is readily digested by a variety of proteolytic bacteria (McAllister et al., 1990), whereas maize has a denser protein matrix (Rooney and Pflugfelder, 1986; Kotarski et al., 1992; Philippeau and Michalet-Doreau, 1997) coupled with the effects of vitreousness (Philippeau and Michalet-Doreau, 1997), which is inversely related to starch digestibility (Correa et al., 2002; Ngonyamo-Majee et al., 2008). These results are contradictory to previous studies reporting a higher starch digestibility of barley compared to maize, and inconsistent with the common ranking of

starch digestibility of cereal grains (wheat, barley, maize and sorghum in descending order) (Herrera-Saldana et al., 1990; Lanzas et al., 2007). Typically, the extent *in vitro* starch fermentation of barley ranges from 87-90% reported by Kotarski et al. (1992), but a lower range of 52-76% has been reported with an average of 67% (Bird et al., 1999). The extent of starch degradability of maize in the current study is still however within the range of 40-77% and 50-90% as reported by Philippeau et al. (1999) and Kotarski et al. (1992), respectively. Other previous studies, Philippeau et al. (1999), Cerneau and Michalet-Doreau (1991) and Herrera-Saldana et al. (1990) reported starch degradability of maize to be 55%, 58% and 62%, respectively. Concurring with previous studies, starch in sorghum was the least digestible and with a slower rate than other cereals as a result of the starch-protein matrix, which is more resistant to moisture, microbial attack and enzyme penetration than that of other grains (Rooney and Pflugfelder, 1986; Theurer, 1986; Herrera-Saldana et al., 1990; Kotarski et al., 1992; McAllister et al., 1993; Al-Rabadi et al., 2009, 2012). Bird et al. (1999) reported an average of 44% for sorghum starch digestibility with a range of 35-51% whereas Herrera-Saldana et al. (1990) reported a higher starch digestibility of 49% as compared to our study. Besides the starch-protein matrix and differences in cell wall architecture, lower starch digestibility in sorghum as compared to other cereals may also be explained by the presence of anti-nutritional factors such as polyphenols, particularly tannins; and phytate (Björck and Nyman, 1987), which may bind to amylase, reducing its activity.

The potentially digestible starch (pdSd) differed among cereal grains ($P < 0.0001$), with wheat showing the highest (94.92%) followed by maize-1 (81.20%), sorghum and maize-2 (74.36 and 74.30%, respectively) and then barley (71.56%) (Figure 3.1). Again, barley was expected to have higher pdSd and lower uS than maize and sorghum because of its highly digestible starch and the fast rate of starch degradation.

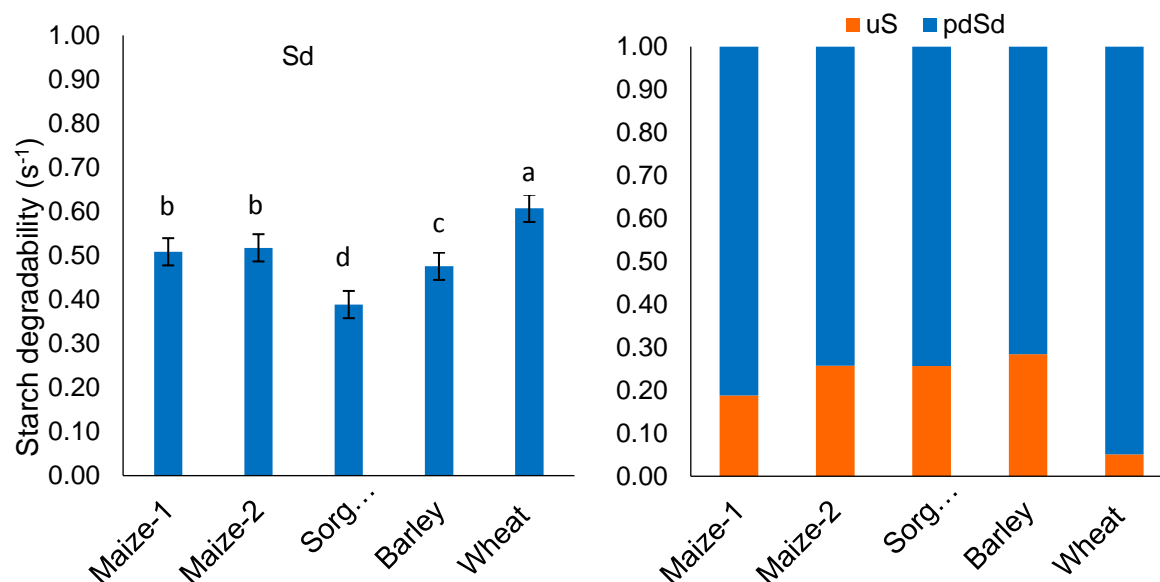


Figure 3. 1. *In vitro* starch degradability (when pooling time-points) and a comparison of potentially digestible starch (pdSd) and indigestible starch (uS) of cereal grains used in this study. Means with different superscript (a-e) differ ($P < 0.05$).

The rates of starch degradation reported in this study differ from those reported in previous studies. For instance, Lanzas et al. (2007) reported fractional digestion rates of starch from an *in vitro* gas production experiment using a 4 mm particle size of 0.15, 0.06, 0.24 and 0.26 h⁻¹ for maize, sorghum, barley and wheat, respectively. In a summary of *in situ* studies, Offner et al. (2003) reported mean k_d values of 0.06, 0.042, 0.35 and 0.33 h⁻¹ for maize, sorghum, barley and wheat, respectively. It is worth noting that the rate of starch digestion in this study was calculated assuming a lag and the presence of indigestible starch (uS), which was lower in wheat (5.08%), followed by maize-1 (18.80%), sorghum (25.64%) and maize-2 (25.70%) and then barley (28.44%) (Figure 3.1). The discrepancies among studies could possibly be explained by differences in methods and estimation of the k_d . *In situ* studies acknowledge the fact that the method tends to overestimate starch degradation rate for rapidly-degrading grains, such as wheat and barley, and underestimates the degradability of slowly-degrading grains, such as maize and sorghum (Offner and Sauvant, 2004). Furthermore, particulate losses through the bag pores and the lower microbial activity inside the bags relative to the rumen may affect the accuracy of estimations (Huhtanen and Sveinbjörnsson, 2006).

Figure 3.2 shows the rate of starch degradability of grains used in the study over a 24 h incubation period. Most of the starch was degraded by 24 h, with wheat having the highest disappearance (97.26%; $P < 0.001$). When results are compared at similar incubation times, sorghum had the lowest values at all incubation times, except for 24 h with 75.92% compared to 74.54 and 77.35% in barley and maize-2, respectively but lower than wheat ($P < 0.001$).

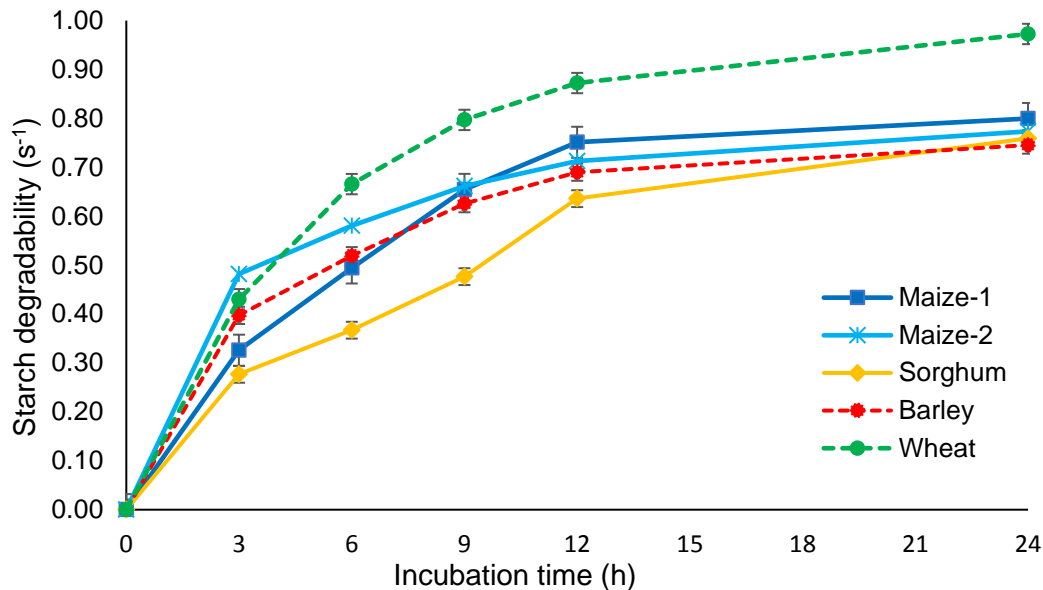


Figure 3. 2. *In vitro* starch degradability of investigated cereal grains over a 24 h incubation time.

Inclusively, article reviews and previous experiments have reported that starch digestibility and degradation rate of cereal grains can be highly variable, and is influenced by numerous factors related to inherent starch architecture and related physicochemical properties as reviewed by Giuberti et al. (2014). Furthermore, discrepancies between studies are expected because of methodological differences such as differences in the proportion of rumen fluid and buffer, type of buffer solution, effects of processing as well as the mathematical models used to evaluate data (Lanzas et al., 2007).

3.4.3 *In vitro* ruminal starch degradability and rate of starch degradation of cereal grains varying in particle size

Table 3.3 and Figure 3.4 show the starch digestibility values, when pooling time points, and respective rates across grains and particle size. Particle size had significant effects ($P < 0.0001$) on starch degradability and rate of starch digestion for all grains, with the starch in small particles being more rapidly digested than starch in larger particles (Figure 3.3). The potentially digestible starch (pdSd) was also affected by particle size ($P < 0.0001$), with the higher potentially digestible starch in smaller particles than the larger particles, whereas indigestible starch (uS) increased with increasing particle size. When pooling time points, the pdSd (uS) was 88.29% (11.71%) for very fine particles, 80.30% (19.70%) for fine particles, 75.36% (24.64%) for medium particles, and 70.72% (29.28%) for coarse particles. The unsieved milled samples had 81.67% pdSd and 18.33% uS.

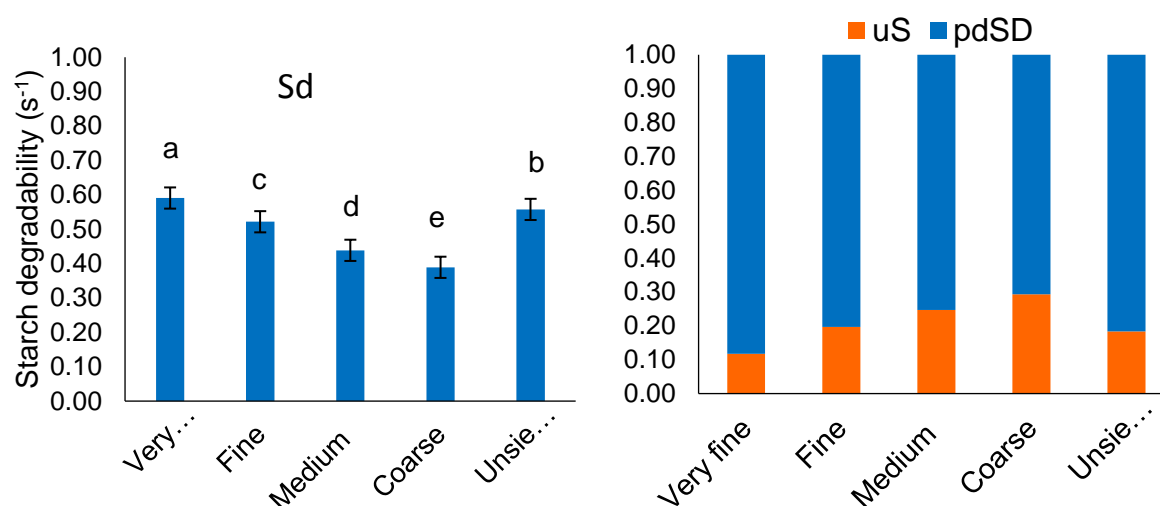


Figure 3. 3. The *in vitro* starch degradability (when pooling time-points) and a comparison of potentially digestible starch (pdSd) and indigestible starch (uS) of particle sizes of the combined cereal grains. Means with different superscript (a-e) differ ($P < 0.05$).

When pooling time points, from the largest to the smallest size fraction, Sd increased from 37.39 to 60.92% for maize-1, from 46.88 to 63.69% for maize-2, from 24.46 and 52.36 % for sorghum, from 40.91 to 58.38% for barley and 56.25 to 71.49% for wheat. The k_d increased from 11.04 to 23.33 h⁻¹ for maize-1, from 18.46 to 28.33 h⁻¹ for maize-2, from 7.65 to 13.84 h⁻¹ for sorghum, from 14.10 to 21.68 h⁻¹ for barley and

from 15.28 to 25.28 h⁻¹ for wheat. These results confirm the inverse relationship between increased particle size and starch digestibility reported in previous studies (Galyean et al., 1981; San Emeterio et al., 2000; Callison et al., 2001; Rémond et al., 2004; Blasel et al., 2006; Al-Rabadi et al., 2009; Mahasukhonthachat et al., 2010; Hoffman et al., 2012; Ferraretto et al., 2013; Giuberti et al., 2014; Gallo et al., 2016). Greater S_d and k_d associated with finer particle sizes is attributed to increased surface area available for bacteria attachment or enzymatic digestion (Huntington, 1997; Ferraretto et al., 2013; Giuberti et al., 2014; Gallo et al., 2016), i.e. a higher degree of access to starch. In comparison, starch degradability of coarser particles is limited primarily by the pericarp, which is mainly resistant to microbial attack (McAllister et al., 1994), and by granulometry (i.e. granule surface structure and/or internal architecture), decreasing accessibility of starch to bacterial and enzymatic digestion (Kotarski et al., 1992; McAllister et al., 1993; Huntington, 1997). It is also possible that the presence of the hard endosperm with a higher protein matrix in the coarser particles and the amylose to amylopectin ratio may have contributed to lower starch degradability in coarser particles. Finer fractions might contain mechanically separated starch granules and protein bodies. They might also contain more floury endosperm, which contains more amylopectin. Amylopectin is more readily digested as compared to amylose due to the fact that amylose has tighter intermolecular bonds between starch molecules (Tester et al., 2004; Stevnebø et al., 2009). The effects of particle size on *in vitro* ruminal starch degradability were more pronounced in sorghum with 28 percentage units (24% of coarse particles and 52% of very fine particles) than for other cereals, possibly due to the extent of particle size reduction during grinding, which may vary among cereal grains.

The k_d values of feeds are incorporated in feed formulation packages such as the Cornell Net Carbohydrate and Protein System (CNCPS). In comparison to the k_d values of our study, the Nutritional Dynamic System (NDS) (CNCPS version 6.5, RUM&N, Reggio Emilia, Italy) has the k_d values in the software's feed database, for finely, medium and coarsely ground maize of 15% h⁻¹, 12% h⁻¹, 10% h⁻¹, respectively, and 10% h⁻¹, 8% h⁻¹, 5% h⁻¹ for sorghum, respectively. Although a general description of particle size of grains (as finely, medium or coarsely ground) is used in modern rationing software to report the k_d, the k_d for medium ground sorghum is similar to our medium particles (8% h⁻¹

¹) and coarsely ground maize is similar to our coarse particles of maize-1 (10% h⁻¹ and 11% h⁻¹, respectively). The NDS feed library k_d value for medium ground barley is 30% h⁻¹, which is higher than our medium particles but similar to our unsieved ground barley. The NDS feed database values for fine and medium ground wheat is 40% h⁻¹ and 35% h⁻¹, respectively which are higher than the results of this study. This confirms the importance of comprehensive analyses of feeds used in specific areas to build reliable feed libraries to be used in precision feeding.

When different fractions are compared with unsieved ground grains for pooled cereals, starch degradability and rate of starch degradation varied. The Sd of unsieved grains was lower than that of very fine particles (55.57 vs. 59.06%) but higher than that of fine (52.16%), medium (43.85%) and coarse particles (38.91%) ($P < 0.0001$). The k_d was generally greater in unsieved than fractions, when pooling cereal grains ($P < 0.0001$), with 24.12% h⁻¹ followed by very fine particles (22.42% h⁻¹), fine particles (19.05% h⁻¹), medium (14.77% h⁻¹) and then coarse particles with 13.31% h⁻¹. This demonstrates how a single characterization (i.e. Sd or k_d) made in an unsieved sample will not be truly representative of the cereal analysed in the laboratory and ultimately fed to the animal. The digestion and the resulting energy and microbial protein will be dependent on the distribution of each particle size and the characteristics of each fraction.

Table 3. 3. Effects of particle size on the starch degradability (Sd) and rate of starch degradation (k_d) of the investigated cereal grains.

		Particle size					SEM	P-value
		Very fine	Fine	Medium	Coarse	Unsieved		
Maize-1	Sd (%)	60.92 ^a	54.37 ^b	45.60 ^c	37.39 ^d	56.01 ^b	0.031	<0.0001
	K_d (% h^{-1})	23.33 ^a	20.21 ^b	16.76 ^c	11.04 ^d	20.75 ^a	0.004	<0.0001
Maize-2	Sd (%)	63.69 ^a	5.94 ^b	52.43 ^b	46.88 ^c	53.34 ^b	0.037	<0.0001
	K_d (% h^{-1})	28.00 ^b	22.16 ^c	17.36 ^d	18.46 ^d	30.35 ^a	0.005	<0.0001
Sorghum	Sd (%)	52.36 ^a	44.58 ^b	31.53 ^c	24.46 ^d	56.24 ^a	0.015	<0.0001
	K_d (% h^{-1})	13.84 ^b	12.18 ^b	8.57 ^c	7.65 ^c	19.43 ^a	0.005	<0.0001
Barley	Sd (%)	58.38 ^a	51.71 ^b	41.82 ^c	40.91 ^c	59.51 ^a	0.015	<0.0001
	K_d (% h^{-1})	21.68 ^b	21.04 ^b	15.33 ^c	14.10 ^c	30.73 ^a	0.005	<0.0001
Wheat	Sd (%)	71.49 ^a	65.76 ^b	59.48 ^{bc}	56.25 ^c	65.29 ^{ab}	0.018	<0.0001
	K_d (% h^{-1})	25.28 ^a	19.66 ^b	15.84 ^c	15.28 ^c	19.35 ^b	0.005	<0.0001

^{a-d} Means within a row with different superscripts differ (P<0.0001)

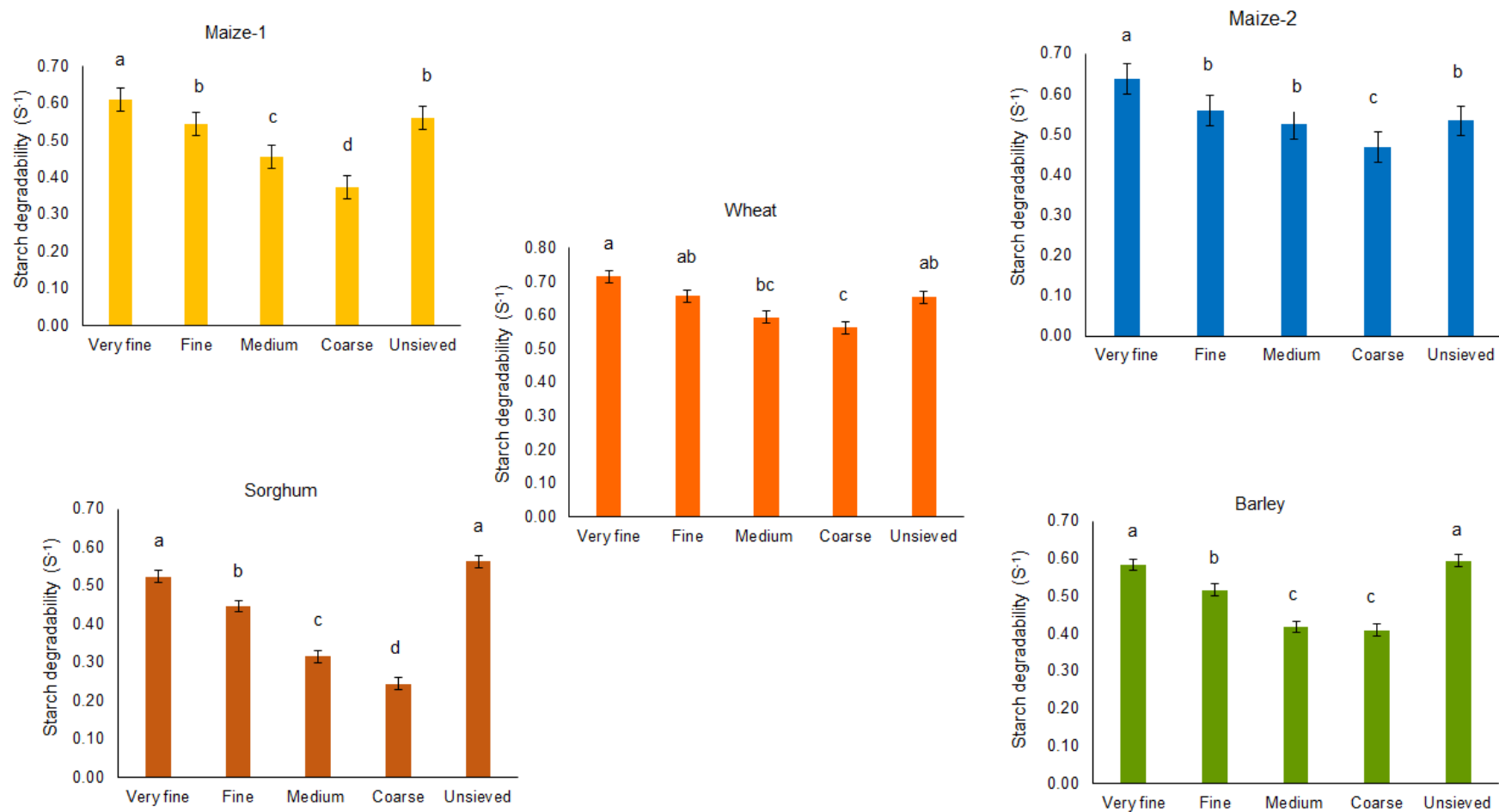


Figure 3. 4. *In vitro* starch degradability of grains varying in particle sizes vs. unsieved grains. Means with different superscript (a-e) differ ($P < 0.05$).

Figure 3.5 shows the effects of particle size on starch degradability, when pooling grains, across time. Finer particles had a higher digestibility than larger particles over time, as a result of a higher degree of access to starch due to the increased surface area per unit volume (Huntington, 1997) and possible other factors, such as presence of the hard endosperms with a more profound protein-matrix and differences in amylose: amylopectin ratios. When results are compared at similar incubation times, starch degradability decreased as particle size increased. These differences were however not always significant. For instance, at 24 h very fine particles showed a significantly higher degradability (87.27%) when compared to coarse particles (70.71%) and medium particles (74.66%) but not significantly different from fine particles (80.64%). Differences are more evident when very fine and fine particles are compared to medium and coarse particle size, at all time points. Coarse particles had the lowest starch degradability throughout the entire incubation period, presumably because starch within the coarse particles is enclosed in the granules within the harder endosperm. As expected, starch degradation increased as the incubation time increased because of increased interactions between substrate and microbial attack.

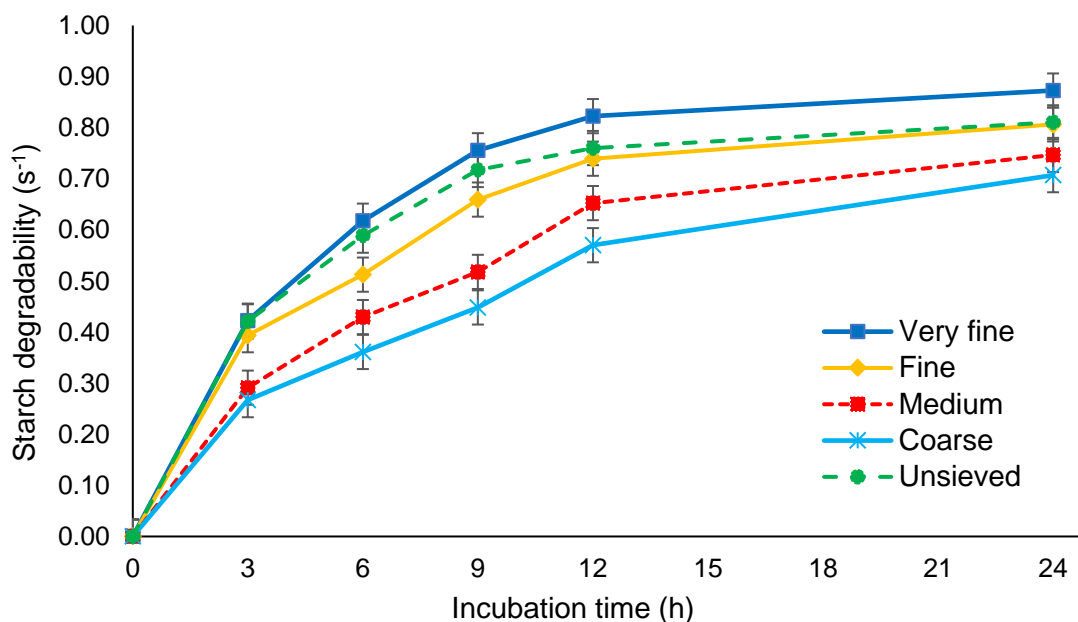


Figure 3. 5. Effects of particle size and unsieved ground grains on *in vitro* starch degradability, when pooling grains, across time.

The interaction between cereal grain x size x time ($P=0.0639$) resulted in the fractions of cereal grains tending to respond differently depending on the incubation time. Table 3.4 shows the starch degradability of sieved fractions (i.e. particle sizes) and unsieved milled grains across incubation times. For all grains, finer particles showed the highest starch disappearance in all assayed time intervals and starch degradation increased as the incubation time prolonged. The starch degradability reached 80% at 12h in very fine particles for both grains.

Table 3. 4. Starch degradability of particle sizes and unsieved ground grains across the 24 h incubation time.

Maize-1		Particle size				SEM	P-value
		Very fine	Fine	Medium	Coarse	Unsieved	
Incubation time, h							
3		40.10 ^a	39.25 ^a	25.12 ^b	22.22 ^b	36.30 ^a	0.033
6		67.52 ^a	49.90 ^{bc}	42.08 ^c	29.85 ^d	57.80 ^{ab}	0.037
9		76.90 ^a	72.52 ^a	57.18 ^b	44.67 ^c	76.13 ^a	0.037
12		83.73 ^a	80.37 ^a	72.38 ^b	57.40 ^c	81.68 ^a	0.037
24		87.55 ^a	81.10 ^{ab}	74.82 ^{bc}	72.80 ^c	83.58 ^{ab}	0.037
Maize-2		Particle size					
		Very fine	Fine	Medium	Coarse	Unsieved	
Incubation time, h							
3		52.83 ^a	48.53 ^{ab}	43.55 ^{bc}	41.00 ^c	55.15 ^a	0.032
6		71.75 ^a	55.13 ^b	54.30 ^b	51.73 ^b	57.33 ^b	0.032
9		78.98 ^a	71.68 ^b	61.25 ^d	53.50 ^e	65.45 ^c	0.032
12		82.75 ^a	75.60 ^b	73.30 ^b	60.70 ^c	63.88 ^c	0.032
24		85.37 ^a	79.97 ^a	80.50 ^a	69.67 ^b	71.25 ^b	0.032
Sorghum							
		Very fine	Fine	Medium	Coarse	Unsieved	
Incubation time, h							
3		31.25 ^a	31.37 ^a	20.32 ^b	14.97 ^b	40.50 ^a	0.049
6		43.63 ^a	41.80 ^a	27.32 ^b	15.25 ^c	55.50 ^a	0.049
9		68.42 ^a	50.07 ^b	29.65 ^c	19.70 ^d	70.43 ^a	0.049
12		81.60 ^a	65.35 ^b	48.40 ^c	44.87 ^c	77.73 ^a	0.049
24		89.75 ^a	78.93 ^b	66.88 ^c	59.70 ^c	84.33 ^a	0.049
Barley							

Table 3.4 continues

	Very fine	Fine	Medium	Coarse	Unsieved		
Incubation time, h							
3	45.12 ^a	45.10 ^a	31.25 ^b	30.87 ^b	46.25 ^a	0.024	<0.0001
6	59.37 ^b	51.80 ^c	42.45 ^d	39.00 ^d	67.03 ^a	0.024	<0.0001
9	75.35 ^a	64.32 ^b	47.72 ^c	49.17 ^c	76.22 ^a	0.024	<0.0001
12	79.62 ^a	69.82 ^b	59.85 ^c	56.97 ^c	78.72 ^a	0.024	<0.0001
24	84.51 ^a	74.89 ^b	67.19 ^c	67.91 ^c	78.21 ^{ab}	0.024	<0.0001
Wheat	Particle size					SEM	P-value
	Very fine	Fine	Medium	Coarse	Unsieved		
Incubation time, h							
3	53.63 ^a	44.42 ^{ab}	37.10 ^b	36.05 ^b	43.96 ^{ab}	0.065	<0.0001
6	78.45 ^a	69.26 ^{ab}	60.28 ^{b^c}	56.46 ^c	68.42 ^{abc}	0.065	<0.0001
9	89.96 ^a	82.77 ^{ab}	74.82 ^{bc}	68.80 ^c	82.11 ^{ab}	0.065	<0.0001
12	95.31 ^a	90.24 ^a	83.98 ^{ab}	76.78 ^b	89.81 ^a	0.065	<0.0001
24	99.77	98.93	93.30	94.43	98.87	0.065	<0.0001

^{a-d} Means within a row with different superscripts differ (P<0.0001)

3.5 Conclusion

In commercial laboratories and as a normal experimental practice, grains are milled using two or more screens of different size to obtain samples of different particles sizes that are then analysed. However, even using a single sieve, different grains result in different mean particle sizes and distributions, which may influence chemical analyses and digestibility results. The objective of this study was to investigate the effects of particle sizes of cereal grains obtained by sieving milled grains on nutritional composition, rumen starch degradation and rate of starch degradation. This study showed that particle size affects the chemical composition, the *in vitro* rumen starch degradability and the rate of starch degradation. Therefore, the results show how various grain particle sizes have the potential to provide the animal with different amounts of nutrients based on composition and starch digestion and possibly resulting in different energy and microbial protein production. Subsequently, the results of this study provide possibilities to better formulate diets for precision starch feeding by incorporating particle size of starch sources as a factor for better characterization of starch. We then propose that the cereal grains fed to high producing ruminants should be sieved and fractions analysed for both composition and rumen degradability to improve their characterization. The improved characterization would then presumably result in a better animal performance prediction. Otherwise if the possibility of feeding various size fractions exist, we will have the possibility of fine tuning the diet and satisfy nutritional requirements for maximum and optimal performance across physiological stages, even when using the same cereal. Every time a new cereal is introduced or when different batches or when a different feed mill is used, variability is added to the ration that could be easily described and ultimately improve animal performance and farm profitability. Moreover, the present finding confirms that starch degradation is inversely related to particle size. Therefore, starch digestion could possibly be shifted post-rumen based on size.

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CHAPTER 4

Short communication: a comparison of two mathematical approaches to predict the rate of starch degradation of different grains and different particle sizes

4.1. Abstract

Starch degradability is often estimated *in vitro* at 7 h and the value is then used to calculate a rate of degradation using a previously published mathematical formula. The objective of this study was to compare mathematical approaches for determining the rate of starch degradation using data from various grains and particle sizes. Three cereals (maize, sorghum and barley) were ground through a 2 mm sieve using a Wiley mill and subsequently sieved to obtain the following sizes: <250, 250-500, 500-1180 and 1180-2000 μm . All fractions, including an unsieved sample, were analyzed for starch and fermented *in vitro* using rumen fluid to quantify starch degradability (Sd). Residual starch of the fermented samples was obtained at 0, 3, 6, 7, 9, 12 and 24 h. Rates of starch degradation (k_d) were calculated with either a first-order decay model (nlin- k_d) using all time points except 7 h, or a mathematical formula using only the 7 h Sd (7 h- k_d). The nlin- k_d 's were used as reference values (observed) and compared with the 7 h- k_d calculated values. Predicted and observed k_d 's were highly correlated across all grains and sizes ($R^2 = 0.94$ to 0.99). Across grains, barley and sorghum resulted in the highest correlation ($R^2 = 0.99$ and $R^2 = 0.98$, respectively) while maize resulted in $R^2 = 0.93$. While observed k_d 's regressed on predicted k_d 's resulted in intercepts not different from zero ($P > 0.44$), slopes were always larger or smaller than 1 ($P < 0.01$) resulting in the 7 h- k_d 's being under- or over-estimated, respectively, compared to the reference nlin- k_d 's. The 7h prediction equation resulted in underestimated values especially for the sorghum and unsieved samples, with differences up to 0.01 h^{-1} . This suggests the need of using a non-linear estimation, using multiple time points, or the development of alternative estimations, especially when quantifying rates of starch degradation for high producing cows.

4.2. Introduction

Starch is the primary source of energy for dairy cows, and characterizing properties of starch sources is of utmost importance to optimize utilization and overall feed efficiency. The rate and extent of starch digestion is highly variable depending on intrinsic as well as extrinsic factors (Patton et al., 2012; Giuberti et al., 2014; Moharrery et al., 2014), and has significant effects on the performance and health of dairy cows. Techniques (*in vitro*, *in situ* and *in vivo*) for evaluating digestion of starch have been developed.

In a review, Huhtanen and Sveinbjörnsson (2006) discussed different methods of measuring ruminal starch degradability (i.e. *in vitro*, *in situ* and *in vivo* techniques) and their respective advantages, challenges and shortcomings. Although *in vitro* starch digestibility has been widely used successfully, it is acknowledged that it cannot provide absolute degradation values required to predict ruminal and intestinal digestibility (Stern et al., 1997; Sveinbjörnsson et al., 2007; Allen and Piantoni, 2014). However, the relative values provided by the *in vitro* method can be related to the actual *in vivo* conditions, therefore, reducing the need of *in vivo* studies for such purpose. It is however very important to obtain reliable *in vivo* reference data. Likewise, a standardized *in vitro* starch digestion method is essential to improve intra-lab repeatability, however this seems to be a challenge (Giuberti et al., 2014).

Owing to the complexity, labour, time and cost of the *in vivo* starch digestibility evaluation, *in vitro* measurements (Tilley and Terry, 1963; Goering and Van Soest., 1970; Menke et al., 1979; Theodorou et al., 1994) are frequently used as alternative procedures to determine starch digestibility in dairy cows (Stern et al., 1997; Giuberti et al., 2014). The *in vitro* starch digestibility method is based on the anaerobic incubation of samples in rumen fluid with buffered medium. Residual starch is then measured to quantify starch disappearance after specific fermentation periods. By using different mathematical approaches, the rate of starch disappearance is then often estimated either using a single or multiple incubation time points (Sveinbjörnsson et al., 2007; Sniffen et al., 2009; Allen and Piantoni, 2014; Giuberti et al., 2014). A 7 h single incubation time has been adopted by most commercial laboratories, as it is considered the mean rumen retention time of concentrates for lactating dairy cows (Giuberti et al., 2014; Gallo et al., 2016). Other time

points, from 0 to 48 h, are used for a supposedly more accurate and precise estimation using various non-linear equations.

To our knowledge, even if widely used, the mathematical formulation using 7 h *in vitro* starch digestibility has not been validated against a reference method. Therefore, the objective of this study was to compare the mathematical approach used for determining the rate of starch degradation using 7 h *in vitro* starch degradation with a non-linear first order decay model used as reference.

4.3. Materials and Methods

Three cereal grains (maize, sorghum and barley) locally sourced (Agricol, Cape Town, South Africa) were ground through either 1- or 2-mm screens using a Wiley mill (Model 4, Thomas-Scientific, USA). Samples were then sieved through a series of sieves to obtain the following sizes: <250, 250-500, 500-1180 and 1180-2000 μm (i.e. very fine, fine, medium, coarse) using a laboratory sieve shaker (Kingtest Laboratory Test Sieve Shaker, Retsch GmbH, Series AS 200 basic, Germany). All samples, including the unsieved sample, were analyzed in duplicate for starch as described by Hall (2009).

All *in vitro* sample residues were analysed for starch to calculate degradability following incubation at 0, 3, 6, 7, 9, 12 and 24 h. All *in vitro* analyses were conducted according to Goering & Van Soest (1970), using 0.25 g of sample in 125-mL Erlenmeyer flasks incubated in a 39.5 °C water bath under constant CO₂ to maintain an anaerobic condition. For the entire experiment, rumen fluid was harvested from the same two lactating Holstein cows in the morning (~07:00) before feeding, at the Welgevallen Experimental Farm of Stellenbosch University. Cows received a total mixed ration containing approximately 38% Lucerne, 7% straw and 63% of a concentrate mixture. The main starch source was maize. Rumen fluid was filtered through two layers of cheesecloth, glass wool, and a double layer of 200 μm mesh before injection into the incubated flasks. Samples were incubated in duplicate at each incubation time. Preliminary observations showed that the majority of the starch disappeared by 24 h without any apparent presence of resistant starch and therefore incubations longer than 24 h were not performed. The residual starch was determined as described by Hall

(2009). All fermentations were completed across 3 runs and samples from each run were considered as experimental replicates.

All procedures were approved by the Research Ethics Committee for Animal Care and Use of Stellenbosch University (protocol number SU-ACUD14-00052).

Calculations, mathematical approaches and statistical analyses

The *in vitro* starch digestibility (ivSd) data obtained at 7 h were used to compute rates of starch degradation for each sample in duplicate according to a formula previously published (Sniffen and Ward, 2011):

$$K_d-7 = (((LN(\text{Starch}, \%DM)) - (LN(((100 - 7h \text{ ivSd}, \%Starch) / 100) * \text{Starch}, \%DM)))) / 7) * 100. \quad (\text{Equation 1})$$

where starch% is the starch content of the grain and ivSd% is the *in vitro* starch degradation as % of starch.

Furthermore, assuming the absence of indigestible starch, the residual starch at time (t) (0, 3, 6, 7, 9, 12 and 24 h) was described by:

$$S(t) = S_{(0)} e^{-k_d(t-L)} + uS; \quad (\text{Equation 2})$$

where $S_{(0)}$ is the size of the digestible soluble and insoluble starch at time 0; k_d is the fractional rate of starch degradation, L is the lag and uS is the estimated indigestible starch. Simultaneous estimations of the parameters k_d and L were initially obtained using PROC NLIN of SAS (SAS Institute, Inc., Cary, NC - USA) and the Marquardt algorithm. The Marquardt algorithm was selected to improve the efficiency of providing least-squares estimation for the non-linear curve fitting approach. Non-linear regression was chosen as the standard procedure because the method assumes equal error at each observation and by simultaneously fitting all parameters to the data, the result provides the smallest residual sums of squared deviations. The necessity of establishing initial parameter values for the non-linear estimations was solved using a linear approach to seed the non-linear estimation as done by Grant and Mertens (1992). The log-linear approach of Van Soest et al. (2005) was used to generate the initial values for each sample to parameterize the decay model.

Rates of degradation from equation 1, using only 7 h *ivSd*, were compared to the non-linear estimation from equation 2 using PROC NLIN in SAS (SAS Institute, Inc., Cary, NC – USA). The goodness of fit was compared using the variance accounted for (R^2) and the residual mean squares (RMS) at convergence, as in Ellis et al. (2005) and Huhtanen et al. (2008), using the average values obtained by pooling all the samples analyzed and then by cereal fraction (all the sieved and unsieved samples). Computations were made as suggested by Piñeiro et al. (2008), with the results of the non-linear estimation being the “observed” values, since it was assumed that those to be the most accurate and precise values. The root mean square errors (RMSE) between the observed and predicted values for each parameter, using the least number of fermentation endpoints, were calculated as follows: $RMSE = \sqrt{\sum (\text{observed} - \text{predicted})^2 / n}$, where n is the number of samples. The mean square prediction error (MSPE) was divided into components resulting from mean bias, slope bias, and random or unexplained variation around the regression line according to analysis of (Theil, 1966) and Bibby and Toutenburg, (1977).

4.4. Results and Discussion

The main objective of the present study was to compare the two mathematical approaches used for determining the rate of starch degradation (k_d). The k_d is typically calculated from *in vitro* starch degradation values using a variety of mathematical approaches. Reliable, precise and accurate predictions of rate of starch degradation is needed to better utilize the feed evaluation systems such as Cornell Net Carbohydrate and Protein System (CNCPS), Nordic Feed Evaluation System (NorFor) and the Nutritional Dynamic System (NDS) and to use the nutrition models and accurately formulate more economical and environmentally friendly dairy rations (Tahir et al., 2013). Therefore, the ultimate goal for using the *in vitro* assay is to simulate the fermentation in the rumen, to produce reliable and accurate estimates to be used in the nutritional models. This reduces the use of animals to obtain needed values. Despite continuous improvement on *in vitro* techniques, a reliable mathematical approach for quantifying the rate of starch degradation (k_d) is very useful because generated k_d 's are an important input in the feed formulation systems and nutrition models such as CNCPS (Van Amburgh et al., 2015). Accuracy and precise determination of k_d is important for more accurate

formulation of dairy rations given the increased focus on precision feeding (Tylutki et al., 2008). It will also increase efficiency of feed utilization and reduce nutrient waste.

Descriptive values of the samples by grains and particle sizes (sieve fractions and unsieved samples) are found in Table 4.1. The starch content varied among cereal grains and particle sizes, with the highest content in maize (77.30%, unsieved) and the lowest in barley (63.33%, unsieved), and were in accordance with their expected ranges reported in previous studies (Herrera-Saldana et al., 1990; Huntington, 1997; Lanzas et al., 2007). Similarly, rumen starch degradability also varied between grains and particles sizes.

Table 4. 1. Mean values of starch, ash and neutral detergent fiber (NDF) (% of DM) and *in vitro* rumen starch degradability of sieved fractions and unsieved ground maize, sorghum and barley.

	Particle size				
	Very fine	Fine	Medium	Coarse	Unsieved
	% of DM				
Maize					
Starch	76.92	75.31	69.93	65.06	77.30
Ash	1.48	1.10	1.12	1.40	1.14
NDF	6.57	10.06	15.64	18.73	11.96
7 h ivSd	72.59	62.96	51.82	44.17	75.75
Sorghum					
Starch	79.02	76.94	75.10	73.66	76.86
Ash	1.48	0.78	1.29	1.25	1.15
NDF	5.50	7.15	12.31	10.42	10.92
7 h ivSd	54.90	43.62	30.81	18.21	69.38
Barley					
Starch	65.35	63.45	57.86	67.17	63.33
Ash	1.76	1.68	2.03	1.65	1.92
NDF	10.73	16.97	29.40	30.25	27.10
7 h ivSd	67.38	58.08	45.10	44.08	71.61

7 h ivSd = *in vitro* rumen starch degradation evaluated after 7 h of rumen incubation, % starch.

The comparison of mean values of fractional rates obtained from the 7 h and non-linear estimation equations are shown in Table 4.2. The values from the non-linear statistical model are considered the “observed values” and are used to compare the 7 h fractional rates. The estimated lags result only from the non-linear model since the 7 h equation (equation 1) does not include any lag estimation nor assumption. The rates of starch degradation calculated from the two mathematical approaches are similar, with very small standard errors. The rates of starch degradation for maize and sorghum are within the expected range, the exception being barley, which was lower than the range

(0.20-0.30 h⁻¹) reported in previous reported results (Offner and Sauvant, 2004; Lanzas et al., 2007). This discrepancy could be attributed to methodological differences, variety of grains, processing of grains, and mathematical models (Lanzas et al., 2007).

Table 4. 2. Comparison of rates (mean \pm SE) of starch degradation as predicted by a 7 h and a non-linear equations per cereal grain, when pooling particle sizes.

Cereal grains	7 h-k _d , h ⁻¹	nlin-k _d , h ⁻¹
Maize	0.1267 (0.0023)	0.1282 (0.0030)
Sorghum	0.0836 (0.0024)	0.0935 (0.0028)
Barley	0.1185 (0.0016)	0.1199 (0.0023)

The relationship between the predicted (7 h-k_d) and observed (non-linear model k_d (nlin-k_d)) is presented in Figure 4.1. The 7 h rates and the estimated values from the non-linear equation were highly correlated ($P < 0.01$), across all grains and sizes, with R^2 ranging between 0.94 and 0.99. Across grains, barley and sorghum resulted in the highest correlation ($R^2 = 0.99$ and $R^2 = 0.98$, respectively) while maize resulted in $R^2 = 0.93$ (Figure 4.2). The t-test resulted in no significant differences between the k_d's. Only the 7 h-k_d's of sorghum tended to be lower than the non-linear k_d's (0.084 vs. 0.094 h⁻¹; $P = 0.44$), with differences of 0.01 h⁻¹, however the correlation was strong ($R^2 = 0.99$). Similarly, the correlation was greater across particle sizes, with unsieved and coarse samples resulted in the highest correlation ($R^2 = 0.95$ and $R^2 = 0.94$, respectively) followed by fine, medium and then very fine particles with $R^2 = 0.90$, 0.89 and 0.87, respectively (Figure 4.3 and 4.4). Overall, this result implies that the two methods produced essentially equal results.

The major differences between these two mathematical approaches (7 h-k_d and nlin-k_d) are the inclusion of *in vitro* incubation time points and lag time in the nlin approach. A 7 h-k_d is determined using a mathematical formula (equation 1) with a single time point (i.e. 7 h) without lag estimations nor assumptions whereas nlin-k_d requires multiple fermentation time points with lag estimations. The primary limitation of the 7 h-k_d approach is the reliance on a single time point.

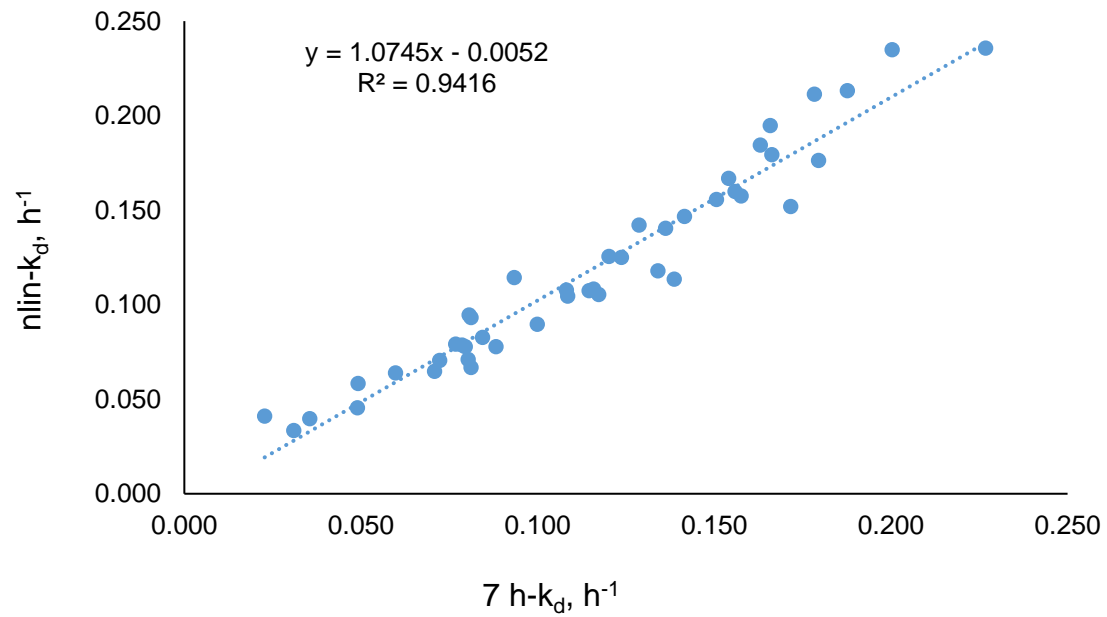


Figure 4. 1. The relationship between the observed ($nlin-k_d, h^{-1}$) and predicted k_d ($7 h-k_d, h^{-1}$) across all grains and particle sizes, estimated from *in vitro* fermentation.

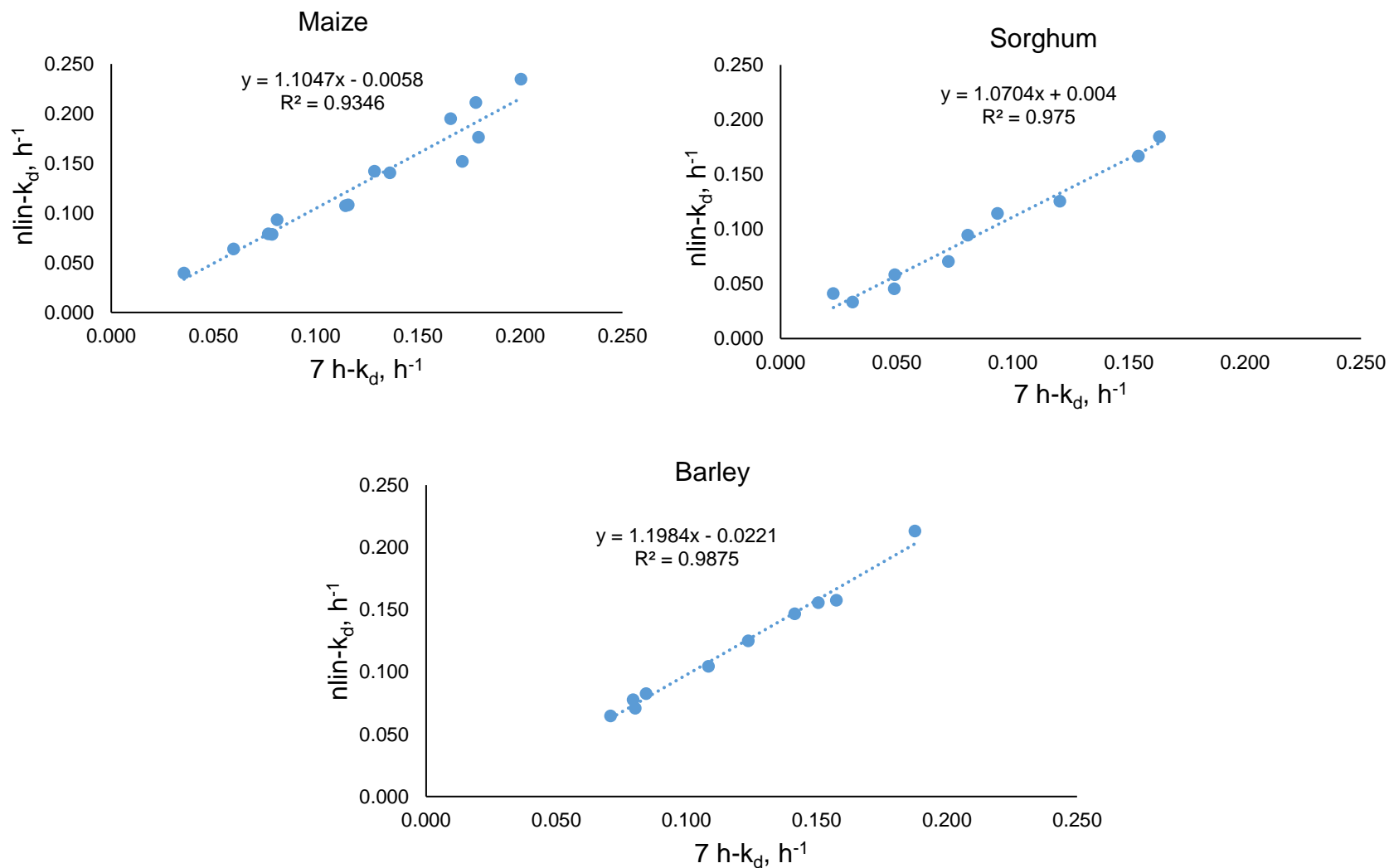


Figure 4. 2. The relationship between the observed ($nlin-k_d, h^{-1}$) and predicted k_d ($7 h-k_d, h^{-1}$) for maize, sorghum and barley, across particle sizes, estimated using in vitro rumen fermentation.

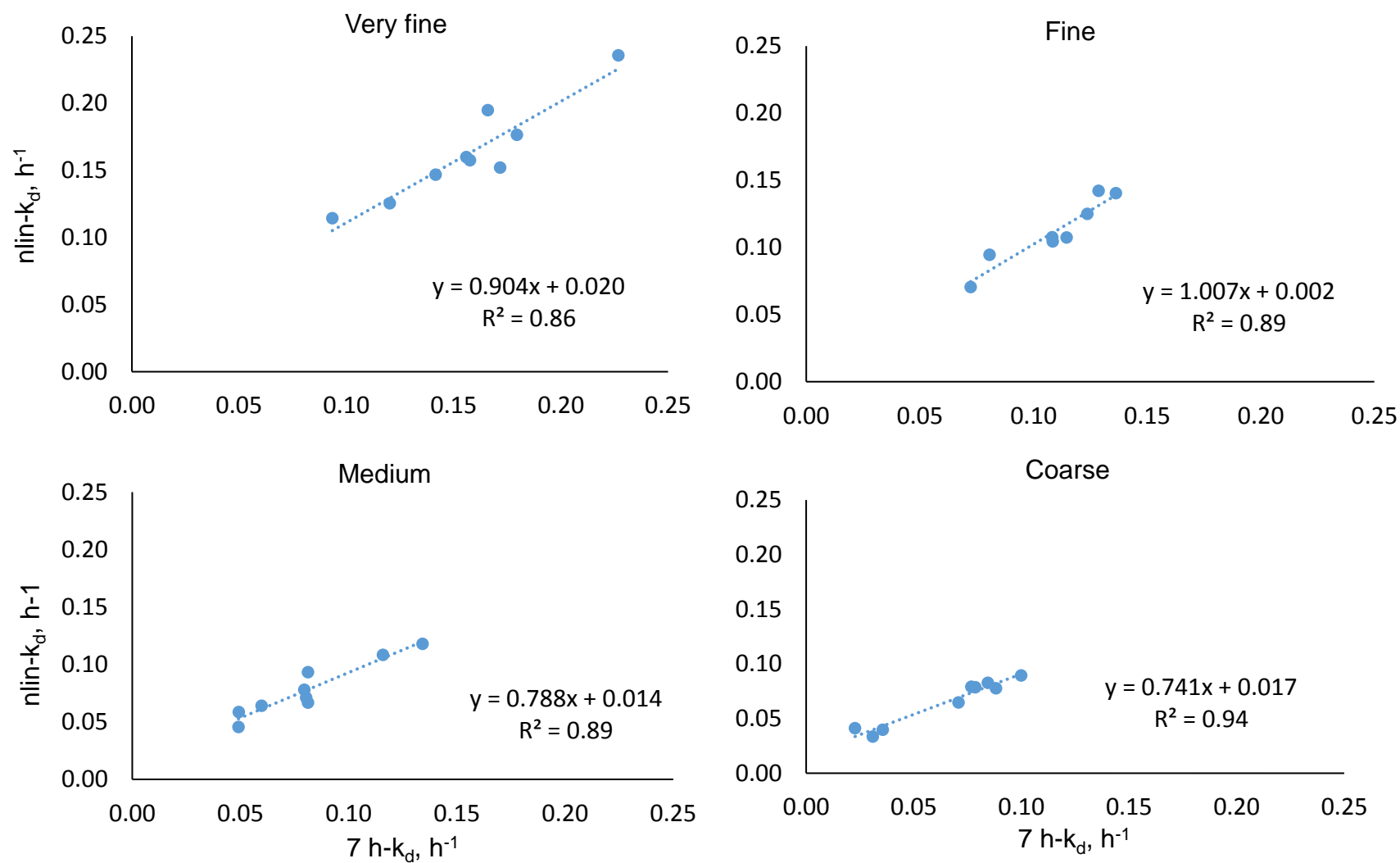


Figure 4. 3. The relationship between the observed (nlin-kd, h⁻¹) and predicted kd (7 h-kd, h⁻¹) for very fine, fine, medium and coarse particle sizes, pooled cereal grains, estimated using in vitro rumen fermentation.

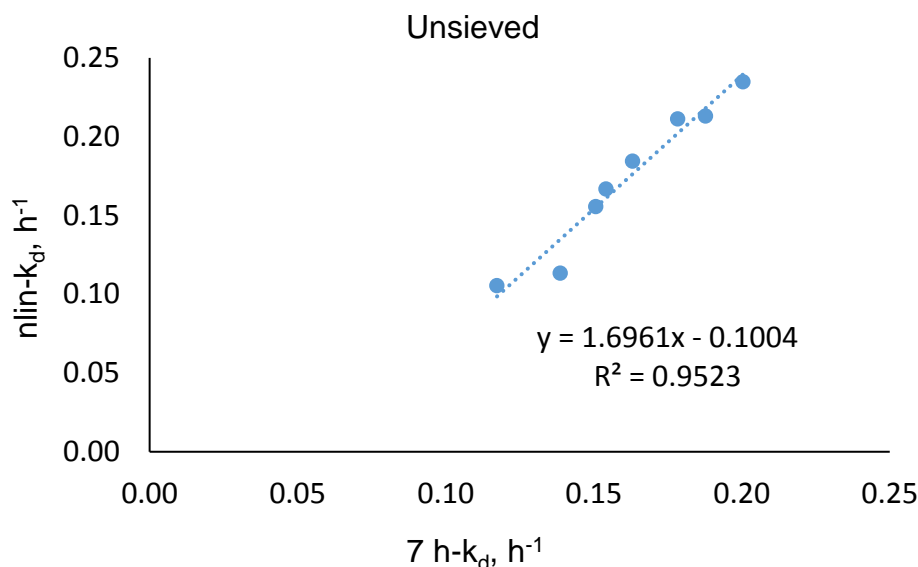


Figure 4. 4. The relationship between the observed ($nlin-k_d, h^{-1}$) and predicted k_d ($7 h-k_d, h^{-1}$) for unsieved samples, pooled cereal grains, estimated using in vitro rumen fermentation values.

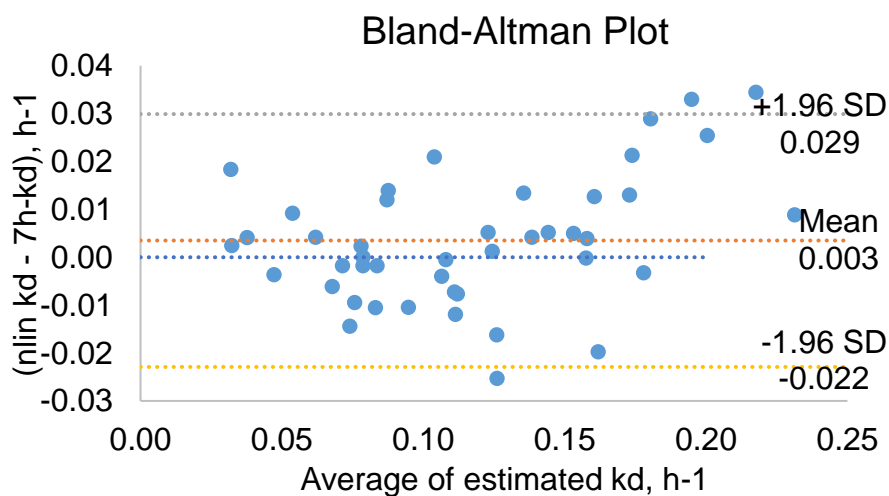


Figure 4. 5. The Bland-Altman plot for the 7 h equation ($7 h-k_d, h^{-1}$) and the non-linear estimation ($nlin-k_d, h^{-1}$).

Although the mathematical approaches estimated the k_d equally well, the slope and intercept were also evaluated for their accuracy. While observed k_d 's regressed on predicted k_d 's resulted in intercepts not different from zero ($P > 0.44$) the slopes were always larger or smaller than 1 ($P < 0.01$) resulting in the $7h-k_d$'s under- or over-estimating the reference non-linear k_d 's. Specifically, the 7 h equation resulted in the highest slope for the unsieved samples (1.70). This slope would result in k_d 's highly underestimated or

overestimated for lower and higher values, respectively. The formula underestimated values especially for the sorghum and unsieved samples, with differences of 0.01 h^{-1} , which, when used in feed formulation programs, may result in overfeeding. The specific relationships between the predicted and observed parameter values are shown in Table 4.3 and 4.4.

Table 4. 3. Comparison of the 7 h (7 h- k_d) and non-linear estimation (nlin- k_d) rates of starch digestion per cereal grains, with pooled particle sizes.

	Maize		Sorghum		Barley	
	7 h- k_d	nlin- k_d	7 h- k_d	nlin- k_d	7 h- k_d	nlin- k_d
K_d, h^{-1}	0.1267	0.1282	0.0836	0.0935	0.1185	0.1199
Variance	0.0023	0.0030	0.0040	0.0028	0.0016	0.0023
Intercept	-0.0127 ^a		0.0040		-0.0221 ^a	
Slope	1.1118 ^b		1.0704		1.1984 ^b	
Correlation	0.96		0.98		0.99	
MSPE	0.0002		0.0002		0.0001	
RSME	0.0073		0.0312		0.0044	

a: Intercept significantly ($P < 0.05$) different from 0. b: Slope significantly ($P < 0.05$) different from 1. RMSE= Root mean squared error. MSPE= mean squared prediction error.

The approaches were evaluated for their prediction accuracy. The regression bias for the equation was tested for its effect on prediction accuracy by regressing the actual values (nlin- k_d, h^{-1}) on predicted k_d (7 h- k_d, h^{-1}). In general, the prediction of the k_d 's resulted in low RMSE for both samples (Table 4.3 and 4.4), indicating a better fit. Across all cereals and fractions, sorghum and unsieved samples had the highest RSME (0,031 and 0,034, respectively). The distribution of the MSPE was similar among predicted k_d 's, ranging from 0.0001 to 0.0002 among cereal grains and particle sizes, the exception being unsieved samples that had the highest MSPE value (0.0006). This result suggests the need of using a non-linear estimation, using multiple time points, or the development of alternative estimations, especially when quantifying rates of starch degradation for high producing cows. The consequence of underestimating the rate of starch degradation in the model is the incorporation of more concentrate and less fibre into the ration leading to potential rumen imbalances and increasing the risk of rumen acidosis.

Table 4. 4. Comparison of the 7 h (7 h- k_d) and non-linear estimation (nlin- k_d) rates of starch degradation per particle sizes and unsieved samples, pooled cereal grains.

	Very fine		Fine		Medium		Coarse		Unsieved	
	7 h- k_d	nlin- k_d	7 h- k_d	nlin- k_d	7 h- k_d	nlin- k_d	7 h- k_d	nlin- k_d	7 h- k_d	nlin- k_d
K_d , h ⁻¹	0.1570	0.1625	0.1155	0.1191	0.0811	0.0781	0.0654	0.0652	0.1613	0.1731
Variance	0.0015	0.0013	0.0008	0.0010	0.0008	0.0006	0.0008	0.0005	0.0007	0.0022
Intercept	0.0207 ^a		-0.0065		0.0141 ^a		0.0168 ^a		-0.1004 ^a	
Slope	0.90375 ^b		1.08768 ^b		0.78842 ^b		0.74126 ^b		1.6961 ^b	
Correlation	0.93		0.97		0.94		0.97		0.98	
MSPE	0.0002		0.0001		0.0001		0.0001		0.0006	
RSME	0.0167		0.0108		0.0092		0.0005		0.0335	

a: Intercept significantly ($P < 0.05$) different from 0. b: Slope significantly ($P < 0.05$) different from 1. RMSE= Root mean squared error. MSPE= mean squared prediction error.

4.5. Conclusion

The *in vitro* fermentation technique has been widely used for past decades in ruminant feed to study feed degradation. The rate of starch degradation is determined from the *in vitro* starch degradation values using published mathematical approaches. In the current study, rates of starch degradation (k_d) were calculated with either a first-order decay model (nlin- k_d) using all time points except 7 h, or a mathematical formula using only the 7 h Sd (7 h- k_d). The nlin- k_d 's were used as reference values (observed) and compared with the 7 h- k_d values. Predicted and observed k_d 's were highly correlated across all grains and sizes. When compared to the observed values from the non-linear procedure in SAS, the 7 h fractional rates were predicted with a different goodness of fit and precision, resulting in different RMSE and MSPE. The 7 h prediction equation resulted in underestimated values especially for sorghum and unsieved samples. It is apparent that the 7 h- k_d approach becomes then a weaker estimation for extreme values, i.e. for very low or very high rates. It seems that for more fermentable starch sources (e.g. very fine grains, high moisture maize or wheat) the 7 h- k_d might underestimate the real k_d , and vice versa. For less fermentable sources, it might overestimate the true k_d . However, differences seem to be small enough to avoid practical issues. One consequence is that when feeding less fermentable starch sources (coarser grains or maize or sorghum) might underfeed and provide less energy. A more problematic consequence is that when using highly fermentable starch sources, we might tend to overfeed that source providing extra energy and possibly creating a risky rumen environment. This result suggests the need of using a non-linear estimation, using multiple time points, or the development of alternative estimations, especially when quantifying rates of starch degradation for high producing cows.

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CHAPTER 5

Effects of a binding agent on *in vitro* rumen degradability of maize and sorghum starch

5.1. Abstract

The objective of the study was to quantify the potential of a starch binding agent (BioProtect™) to reduce *in vitro* rumen starch degradation of maize and sorghum particles varying in size. Maize and sorghum grain samples were ground through 1- and 2-mm sieves using a Wiley mill and subsequently sieved to obtain the following sizes: <250, 250-500, 500-1180 and 1180-2000 µm (i.e. very fine to coarse). All fractions were separately analyzed for starch. Samples were treated 24 h before fermentation by spraying with BioProtect™ according to product guidelines. Both, treated and untreated maize and sorghum samples, were fermented *in vitro* for 0, 6, 12 and 24 h to quantify starch degradability (Sd). Rates of degradability (k_d) were calculated using a first order decay model and 48 h fermentation residuals were used to estimate indigestible starch. As particle size decreased, starch increased from 701 to 821 g/kg and from 730 to 810 g/kg for maize and sorghum, respectively. For both grain types, Sd and k_d increased linearly with decreased particle size. The Sd (k_d) increased from 41 (0.10) to 58% (0.26) and from 30 (0.11) to 53% (0.24 h⁻¹) for maize and sorghum, respectively. BioProtect™ was effective in decreasing starch degradability for both grains. The product was more effective with smaller particle size, by reducing Sd 17%-units for the smallest particles vs. 7%-units for the largest particles. A time interaction was observed, showing that the highest impact of BioProtect™ occurred after 12 h of fermentation for both grains. The starch binding agent resulted in an effective decrease of *in vitro* starch degradation, but results would be affected by particle size and fermentation time. Simulations with the NDS software (CNCPS version 6.5, RUM&N, Reggio Emilia, Italy) were done to evaluate the effects of BioProtect™ treated or untreated maize and sorghum varying in particle sizes on starch digestibility, energy and microbial protein estimations, assuming the requirements of a high-yielding lactating cow and a standard TMR with at least 50% forage. Simulations showed how the use of BioProtect™ can reduce rumen starch

digestibility, thereby possibly increasing rumen starch escape and post rumen starch digestibility. The simulated total tract digestibility was not decreased by the use of BioProtect™, but microbial protein production was slightly reduced. Although the results showed positive effects of BioProtect™ on reducing rumen starch degradation, in our simulations, larger particles were more effective at shifting site of digestion and it could therefore be a more cost-effective option, assuming a consistent total tract starch digestibility.

5.2. Introduction

Maize and sorghum are widely used in the dairy industry and represent a major source of energy. They consist on average of 72 to 76% starch with ≤ 60 to $\geq 80\%$ of starch fermented in the rumen (Nocek and Tamminga, 1991; Huntington, 1997). Ruminal starch digestibility is influenced by intricate interrelations among several intrinsic and extrinsic factors such as the starch-protein matrix within the endosperm of grains, the type of processing of the grains, the diet composition and animal factors, among others, as extensively documented (Firkins et al., 2001; Huntington and Huntington, 2006; Ferraretto et al., 2013; Giuberti et al., 2014). Starch that is ruminally-fermented results in the production of volatile fatty acids (VFA), primarily propionate, and microbial protein. However, rapidly and/or excessive ruminal fermentability can cause a severe drop in ruminal pH, resulting in ruminal acidosis, decreased fibre digestibility and efficiency of microbial protein production (Firkins et al., 2001), reduced feed intake (Allen, 2000), altered rumen biohydrogenation (Bauman and Griinari, 2001), and ultimately decreased milk production. Starch that escapes to the small intestine is enzymatically digested resulting in the production of glucose or fermented in the hindgut to VFA. All non-fermented starch as well as non-absorbed glucose will be excreted together with microbial biomass synthesised in the hindgut (Reynolds, 2006).

The rate, site and extent of starch digestion have a profound effect on the amount and profile of substrates (e.g. acetate, propionate, lactate, glucose) available to the cow, hence great effects on lactation performance (Nocek and Tamminga, 1991; Allen, 2000). The optimal site of starch digestion is still unclear, as controversy exists on the benefits

and detriments of ruminal versus post-ruminal starch digestion (Owens et al., 1986, 2016; Firkins et al., 2001; Taylor and Allen, 2005; Reynolds, 2006). Production studies provide no clear evidence that post-ruminal starch digestion increases milk yield or milk composition as compared to ruminal starch digestion (Nocek and Tamminga, 1991; Reynolds, 2006). However, the potential benefits of starch digestion in the small intestine, as compared to the rumen, include a higher energy efficiency (30 to 42% higher; Owens et al., 1986) leading to higher efficiency of metabolisable energy utilization, reduced risk of rumen acidosis and depression of feed intake, increased ruminal fibre digestion, and increased supply of glucogenic substrates (Svihus et al., 2005; Huhtanen and Sveinbjörnsson, 2006). Moreover, shifting the site of starch digestion to the small intestine, is suggested to increase milk protein production by sparing amino acids from being used for gluconeogenesis in the liver (Huhtanen and Sveinbjörnsson, 2006). Larsen et al. (2009) reported that feeding diets that partly shift the site of digestion from the rumen to the small intestine is an attractive strategy to overcome some of the nutritional shortcomings associated with meeting the nutrient demands of transition cows.

Traditionally processing of maize and sorghum has focused on increasing rumen digestibility in an effort to meet the energy demand of high producing dairy cows in order to optimise milk income over feed costs and efficiency. However, during specific physiological stages, decreasing rumen digestion and shifting site of digestion may be useful given the reasons aforementioned. There have been several studies on the potential of shifting the site of starch digestion to the small intestine by using different starch sources (Herrera-Saldana et al., 1990; McAllister et al., 1990; Taylor and Allen, 2005; Mikula et al., 2011) or processing methods (Beauchemin et al., 2001; Callison et al., 2001; Rémond et al., 2004; Larsen et al., 2009) or by chemical treatments (e.g. sodium hydroxide (NaOH) and formaldehyde (HCHO); Deckardt et al., 2013)). It has been, however, noted that strategies aimed at shifting the site of digestion of starch from the rumen to the small intestine will only be successful if starch is extensively digested in the small intestine (Bird et al., 1999).

Recently, there has been an interest in a product named BioProtect™ (Realistic Agri, Ironbridge, UK), a starch binding agent. The active ingredient in BioProtect™ is a stable non-volatile organic salt that complexes with the hydroxyl groups of starch at

neutral or slightly acidic conditions (pH 6 to 7), as observed in the rumen (Dunshea et al., 2012). These complexes decompose under more acidic (pH 2 to 3) conditions as in the abomasum and duodenum, making the starch available for enzymatic digestion (Dunshea et al., 2012). BioProtect™ can allow for greater post-ruminal enzymatic digestion of starch (Dunshea et al., 2012, 2013) by means of reducing the rate of rumen starch fermentation (Gonzalez et al., 2014). Therefore, BioProtect™ has the potential to improve utilization of highly fermentable starch such as wheat by shifting the site of starch digestion from the rumen to the lower intestine, consequently reducing the risk of rumen acidosis (Dunshea et al., 2013). However, Gonzalez et al. (2014) recommended further *in vitro* and *in vivo* studies to confirm these claims. More recently, Van Zyl (2017) investigated the efficiency of the same product (BioProtect™) on *in vitro* fermentation kinetics and starch digestibility in dairy cows. BioProtect™ was sprayed on maize samples at 10 ml per kg (equivalent to 10 L/tonne). Van Zyl (2017) reported that the treatment of maize with BioProtect™ did not significantly affect *in vitro* gas production or starch disappearance of maize. Nonetheless, numerical tendencies were observed with the use of a starch binder. In another *in vitro* trial, Van Zyl (2017) showed a positive effect of the starch binder with coarsely ground (4-mm screen) low vitreous maize but it did not affect the *in vitro* starch disappearance of finely ground (1 mm) low vitreous maize. In the *in vivo* trial, Van Zyl (2017) ground maize using a 4-mm screen, which is commonly used in the South African feed industry, and hypothesised that BioProtect™ would decrease starch digestion in the rumen, making more starch available for digestion in the small intestine and thereby increasing total tract starch digestion in dairy cows. The results showed that BioProtect™ decreased total tract starch digestibility of maize (94.47 vs. 91.47%), contrary to Gonzalez et al. (2014), who reported that BioProtect™ does not reduce whole tract starch digestibility of wheat in sheep. Although these authors used different starch sources, the effectiveness of BioProtect™ is still not clear.

The ultimate goal of mechanical processing of grains is to reduce the particle size, increasing the surface area, making the starch of the grain more accessible for microbial attack and enzymatic digestion (Huntington, 1997; Offner et al., 2003; Giuberti et al., 2014; Gallo et al., 2016), thereby enhancing the rate and extent of starch digestion (Ferraretto et al., 2013). It is therefore expected that a reduction of particle size will

increase the accessibility and interaction between the starch and BioProtect™, thereby protecting starch from degradation in the rumen. Unexpectedly, BioProtect™ was effective on 4 mm ground maize but not on 1 mm ground maize (Van Zyl, 2017). These results were not expected given that a higher degree of processing exposes finer granules of amylopectin and amylose for BioProtect™ attachment (Van Zyl, 2017). It has been reported that increasing mean particle size is inversely related to starch digestibility (Ferraretto et al., 2013; Gallo et al., 2016), primarily due to an increase in surface area exposed to microbial and enzymatic attack. Moreover, Al-Rabadi et al. (2009) demonstrated that the fractional-digestion rate, as a function of the fragment size, is controlled by diffusion of enzyme through the grain fragment.

There are limited studies on BioProtect™ and previous *in vitro* studies that investigated the effects of BioProtect™ were carried out using the *in vitro* gas production technique to determine starch degradation. The shortcoming of the *in vitro* gas production technique as compared to the *in vitro* rumen fermentation technique by Goering and Van Soest (1970) is that it does not measure residual starch, as a result conclusions are based on the whole grain and not particularly on starch. Moreover, a rapidly fermentable starch source i.e. wheat was used in previous studies, with the exception of Van Zyl (2017), where maize was tested. Most (if not all) previous studies focused on processing of grains simply by grinding grains with different sieves and therefore reducing particle size but also obtaining a variable distribution of particles, which can affect starch digestibility (Huntington, 1997; Firkins et al., 2001). Within grain type, different fragment size (sieve fractions) possess different surface area affecting the magnitude of starch digestion (Al-Rabadi et al., 2009, 2012). The effects of the starch binding agent on maize and sorghum of different particle sizes is therefore necessary to clarify the effects of BioProtect™. Therefore, the objective of this study was to quantify the potential of a starch binding agent (BioProtect™) in reducing *in vitro* rumen starch degradation of maize and sorghum particles varying in size. A further objective was to simulate the effects of BioProtect™ treated or untreated maize and sorghum varying in particle size on starch digestibility, energy and microbial protein estimations of a high-yielding lactating cow using the NDS software (CNCPS version 6.5, RUM&N, Reggio Emilia, Italy).

5.3. Materials and Methods

5.3.1 Treatment of maize and sorghum with BioProtect™

Whole maize and sorghum grains, obtained from a local commercial supplier (Agricola, Cape Town, South Africa), were ground with a 1- or 2-mm sieve using a Wiley mill (model 4, Thomas-Scientific, USA). Ground cereals were then sieved, using a laboratory sieve shaker (Kingtest laboratory test sieve, Retsch GmbH, Series AS 200 basic, Germany), to obtain 4 different subsamples for each cereal with different particles' size: <250, 250-500, 500-1180 and 1180-2000 µm. Samples were treated at least 24 hours before fermentation with BioProtect™, by spraying according to the product guidelines (8 L/tonne of cereal grain). Both treated and untreated maize and sorghum were used to determine the *in vitro* starch degradability (ivSd).

5.3.2 *In vitro* starch degradability

In vitro rumen degradability was carried out as described by Goering and Van Soest (1970), with slight modifications. Sample of approximately 0.25 g was weighed into 125-ml Erlenmeyer flask prior to the addition of 40 ml of buffer prepared according to Goering and Van Soest. (1970). Erlenmeyer flasks were placed in the water bath preheated at 39.5°C, closed with rubber stoppers and flushed with CO₂ to reduce the medium, pending incubation with rumen fluid.

Rumen fluid was harvested, at Welgevallen Experimental Farm of the Stellenbosch University, from two ruminally cannulated lactating Holstein-Friesian cows around 07:00, before the morning feeding, and mixed. Cows were fed twice a day (07:30 and 17:00) a total mixed ration (TMR) containing approximately 30% Lucerne, 7% straw and molasses and 63% concentrate mixture on dry matter (DM) basis. Maize was the main starch source. Rumen fluid was obtained directly from the ventral area of the rumen by hand using a cup and kept in a pre-warmed thermos flask until use (within approximately 20 minutes of harvest). In the laboratory, the rumen fluid was filtered through two layers of cheesecloth, glass wool, and a double layer of 200-µm porosity mesh into a 1-L Erlenmeyer flask pre-warmed at 39.5°C. It was then flushed with CO₂, to purge out air, before injecting 10 ml in each incubated flask using a pre-warmed two-ring syringe (Dosys™, Socorex, Switzerland). Residual starch was quantified at 0, 6, 12 and 24 h to

determine starch degradation and rate of starch degradation. Undigested starch at 48 h (uS) was utilized to estimate indigestible starch. An anaerobic condition was maintained throughout the incubation period by continuous gassing with CO₂ using a pressurized CO₂ manifold connected to each flask. Flasks were checked for leaks, and gently swirled at regular intervals during incubation. At each incubation time, corresponding flasks were removed and plunged in an ice bath to stop microbial activity and fermentation. The residual starch was determined by using a thermostable α -amylase and amyloglucosidase as described by Hall (2009). Three runs were conducted for each cereal, in duplicate of each sample for each incubation time. The same cows and rumen fluid collection time were used for the entire experiment.

All procedures of this experiment were approved by the Stellenbosch University Research Ethics Committee for Animal Care and Use (protocol number SU-ACUD14-00052).

5.3.4 Chemical analysis

All particle size fractions were separately analysed for starch as described by Hall (2009), with slight modifications. Briefly, sample of approximately 0.25 g was weighed into a 50 ml plastic test tube prior to adding 30 ml of 0.1M sodium acetate buffer solution (pH 5) containing 300 μ L of thermostable α -amylase (ANKOM Technology Corp.; α -amylase, heat stable) to each test tube. Samples were incubated in a 100°C water bath for an hour to gelatinise and partially hydrolyse starch. Samples were then cooled to 50°C before adding 300 μ L of amyloglucosidase (AEB Africa, South Africa), and incubated at 50°C for 2 h to completely hydrolyse starch into glucose. The entire sample content was then filtered through glass wool on a funnel into a 100-ml volumetric flask and the filtrate was brought to volume with distilled water. Volumetric flasks were gently shaken to mix the content, then 1 mL aliquots were transferred to 2.0 mL microtubes (Lasec, South Africa) and centrifuged for 10 min at 1000 x g. For absorbance, 300 μ L GOPOD reagent solution was added to a microplate. A glucose standard curve was prepared at the concentration of 0, 2, 4, 6, 8, 10 μ L of D-glucose standard solution (GOPOD, Megazyme, Wicklow, Ireland). The 10 μ L aliquots were then added to the microplate with the GOPOD reagent solution, incubated at 50°C for 20 min and absorbances were immediately

measured using a spectrophotometer (Spectrostar nano, BMG Labtech) at 505 nm wavelength. The standard curve was used to determine the concentrations of glucose, which were then used to calculate the starch content of the samples. For fermented samples, the starch content was analysed by using the entire content of samples in Erlenmeyer flasks (i.e. residue + medium) and therefore soluble starch was included in the resulting starch content. All chemical analyses were done in duplicate.

5.3.5 Calculations and statistical analysis

Rates of starch degradation were computed using a first order decay model according to the following equation:

$$S(t) = S_{(0)} e^{-k_d(t-L)} + uS;$$

where $S_{(0)}$ is the size at time 0 of the soluble and insoluble starch; k_d is the fractional rate of starch digestion, L is the lag and uS is the estimated indigestible starch. Simultaneous estimations of the parameters k_d and L were obtained using PROC NLIN of SAS (version 9.3; SAS Institute, Inc., Cary, NC) and the Marquardt algorithm. The Marquardt algorithm was selected to improve the efficiency of providing least-squares estimation for the non-linear curve fitting approach. Non-linear regression was chosen as the standard procedure because the method assumes equal error at each observation and by simultaneously fitting all parameters to the data. The result provides the smallest residual sums of squared deviations. The necessity of establishing initial parameter values for the non-linear estimations was solved using a linear approach to seed the non-linear estimation as done by Grant and Mertens (1992). The log-linear approach of Van Soest et al. (2005) was used to generate the initial values for each sample to parameterize the decay model.

In vitro starch degradability values and the rates estimated by the nonlinear regressions were analysed as response variables by the GLIMMIX procedure of SAS (version 9.3; SAS Institute, Inc., Cary, NC) using a factorial arrangement of particle size, cereal, BioProtect™, time (for Sd only) and respective interactions. Fermentation run ($n = 3$) was considered as a random effect. Differences between means were declared significant at $P \leq 0.05$ using the LSMEANS and the Tukey adjustment. Trends were

considered for statistical differences between $0.05 < P \leq 0.10$. Results are reported as LSMEANS.

5.4. Results and Discussion

5.4.1. Effects of varying particle sizes on *in vitro* starch degradability

As particle size decreased, starch content increased from 701 to 821 g/kg, for maize, and from 730 to 810 g/kg, for sorghum. For both grain types, starch degradability (S_d) and rate of starch degradation (k_d) increased linearly with decreased particle size ($P < 0.01$). Both S_d and k_d , when pooling time points, increased in fact from 41 and 58% and 10 to 26.0 h^{-1} respectively for maize. The equivalent values for sorghum increased similarly from 30 to 53% for S_d and from 11 to 24.0 h^{-1} for k_d receptively (Table 5.1). In agreement with previous studies, starch degradation was inversely related to particle size (Galyean and Owens, 1981; San Emeterio et al., 2000; Callison et al., 2001; Rémond et al., 2004; Blasel et al., 2006; Al-Rabadi et al., 2009; Mahasukhonthachat et al., 2010; Ferraretto and Shaver, 2012; Hoffman et al., 2012; Giuberti et al., 2014; Gallo et al., 2016). Decreasing the particle size increases the surface area available for bacteria attachment or enzymatic digestion (Huntington, 1997; Ferraretto et al., 2013; Giuberti et al., 2014; Gallo et al., 2016), resulting in an increased rate and extent of starch degradation (Nocek and Tamminga, 1991). Blasel et al. (2006) showed that for each 100 μm increase in mean particle size in ground maize grain, the degree of starch access by α -amylase decreased by 26.8 g/kg starch. Cerneau and Michalet-Doreau (1991) reported that, when mean ground maize particle size increased from 0.8 to 6.0 mm, starch degradability decreased from 57.8 to 44.0%. More recently, Gallo et al. (2016) reported a decrease of 6.3% starch degradability for each 1-mm increase in mean particle size of maize. In this study k_d decreased linearly ($-4.9 h^{-1}$) for each 1-mm increase in mean particle size. Although there are notable differences in the starch values of those studies, it is important to underline that besides physiochemical properties of grains that can influence starch degradation as extensively reviewed (Tester et al., 2004; Svihus et al., 2005; Giuberti et al., 2014) Differences in *in vitro* techniques (such as source of rumen fluid, diet of the source of rumen fluid, substrate to rumen fluid plus buffer ratio, processing of grains) can also influence starch degradability (Giuberti et al., 2014). In addition to the

surface area effects, starch within coarse or large particles is harbored in granules within the endosperm, still protected by the pericarp, which is the foremost barrier, limiting microbial accessibility to starch granules (Huntington, 1997), hence reducing starch degradability.

Table 5. 1. Effects of particle size on the rate and extent of degradability of maize and sorghum starch.

		Particle size				SEM	P-value
		Very fine	Fine	Medium	Coarse		
Maize	Sd (%)	58.20 ^a	53.55 ^b	49.29 ^c	41.75 ^d	0.045	<.0001
	K_d (h^{-1})	26.4 ^a	23.0 ^b	19.0 ^c	10.8 ^d	0.021	<.0001
Sorghum	Sd (%)	53.78 ^a	47.08 ^b	40.52 ^c	30.45 ^d	0.046	<.0001
	K_d (h^{-1})	24.4 ^a	14.1 ^b	14.8 ^b	11.2 ^c	0.041	<.0001

^{a-d} means within a row with different superscripts differ ($P < 0.05$)

5.4.2. Effects of Bioprotect™ on starch degradability of maize and sorghum varying in particle sizes

The study was conducted to quantify the potential of a starch binding agent (BioProtect™) in reducing *in vitro* rumen starch degradation of maize and sorghum particles varying in size. Overall, BioProtect™ was effective ($P < 0.001$) in decreasing starch degrading for both maize and sorghum grains (Table 5.2). In maize, when pooling time and size, the extent of starch degraded decreased from 56 to 46% and from 48 to 37% in sorghum. When pooling particle size, the k_d decreased from 25.0 to 14.0 h^{-1} and 19.0 to 13.0 h^{-1} for maize and sorghum, respectively. Dunshea et al. (2012) investigated the effect of a starch binding agent on the rate of *in vitro* gas production of wheat ground to pass through a 1-mm sieve. Untreated maize was also included in the study. Dunshea et al. (2012) found that the maximum gas production was higher for untreated wheat as compared to BioProtect™-treated wheat and untreated maize (134 vs. 129 and 130 mL/g, respectively, $P = 0.05$). Moreover, the rate constant was found to be greater for untreated wheat than treated wheat, which was in turn greater than the untreated maize (0.267 vs. 0.207 and 0.173 min^{-1} , $P < 0.001$). In that case, wheat fermented faster than maize and BioProtect™ decreased the *in vitro* rate of fermentation of wheat (Dunshea et al., 2012).

The active ingredient in BioProtect™ is a stable non-volatile organic salt that, according to the suppliers, complexes with the hydroxyl groups of starch at neutral or slightly acidic conditions (pH 6 to 7), as observed in the rumen (Dunshea et al., 2012). These complexes decompose under more acidic (pH 2 to 3) conditions as in the abomasum and duodenum, making the starch available for enzymatic digestion (Dunshea et al., 2012). Consequently, the product (BioProtect™) is claimed to increase the digestibility of starch by means of reducing the rate of rumen starch fermentation and increasing the rate of post-ruminal starch utilization. The results of this study demonstrate that BioProtect™ slows the fermentation of both grains, in agreement with Dunshea et al. (2012). BioProtect™ decreased the S_d and k_d of grains by 10%-units and by 8%-units ($P < 0.0001$), respectively. Dunshea et al. (2012a) reported a decrease of 6%-units for the rate of starch fermentation for wheat treated with the starch binding agent. Interestingly, Van Zyl (2017) reported that BioProtect™ was not effective to decrease *in vitro* starch fermentation when maize was milled through a 1-mm screen, irrespective of vitreousness but effective on low vitreous maize milled using a 4-mm screen. In the same study, BioProtect™ decreased the rate of starch digestion of 4-mm ground low vitreous maize by 4%-units. The variability in the response between these studies could possibly be explained by differences in techniques, methodology, grains, degree of processing of grains, the amounts and process of BioProtect™ incorporation. According to Dunshea et al. (2013), BioProtect™ decreases the rate of starch rumen fermentation of wheat in a dose-dependent manner (0, 4, 8 and 16 ml/kg), with response maximised at 8 ml/kg, hence the product guideline of application rate of 8 L per tonne.

Table 5. 2. Effects of BioProtect™ on the rate and degradability of maize and sorghum starch.

		Untreated	BioProtect™-treated	SEM	P-value
Maize	S_d (%)	55.65 ^a	45.75 ^b	0.044	<0.0001
	K_d (h^{-1})	25.2 ^a	14.4 ^b	0.021	<0.0001
Sorghum	S_d (%)	48.44 ^a	37.47 ^b	0.046	<0.0001
	K_d (h^{-1})	18.8 ^a	13.5 ^b	0.041	<0.0001

^{a-b} means within a row with different superscripts differ ($P < 0.05$)

No interaction was observed between cereal and BioProtect™, indicating that the binding agent affected *in vitro* starch degradation of both grains in a similar manner. When the results are compared at different particle sizes, the product was more effective with smaller particle size, reducing Sd by 17%-units for the smallest particles vs. 7%-units for the largest particles. This result implies that the binding agent is more efficient with finer particles where starch is highly degradable, with disrupted protein matrix and disorganized starch granules. It could also be due to the accessibility of starch and its interaction with the product (BioProtect™). The product was therefore able to bind more efficiently to starch in finer particles, inhibiting its fermentation.

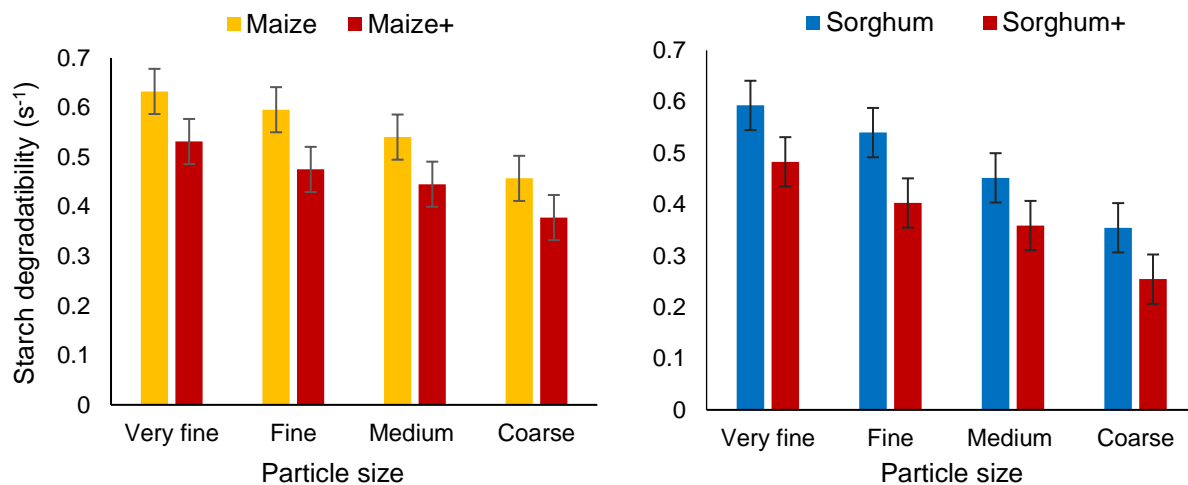


Figure 5. 1. Effect of BioProtect™ on maize and sorghum starch degradation of varying particle size.

According to Al-Rabadi et al. (2009) starch granules in particles larger than 500 µm will predominantly be within intact cell walls, whereas particles smaller than 250 µm would be expected to have many broken cells with exposed intra-cellular content. Besides, BioProtect™ had more access to starch in finer particles, possibly due to the surface area available for reaction between BioProtect™ and starch, making starch more exposed for Bioprotect™ contact. The effects of Bioprotect™ on maize and sorghum starch degradation of varying particle sizes are presented in Figure 5.1, with respective k_d values in Table 5.3. It was also observed that increasing particle size seems to have a larger effect on decreasing rumen starch digestion than the BioProtect™ (Figure 5.1 and

Table 5.3). However, increased particle size may have a negative effect on total tract Sd (Ferraretto et al., 2013) because coarse particles are likely to pass through the intestines without being digested (Fredin et al., 2014).

Table 5. 3. Effect of BioProtect™ on rate of starch degradation (k_d , h^{-1}) of varying particle size

	Particle size				SEM	P-value
	Very fine	Fine	Medium	Coarse		
Maize	32.20 ^a	31.64 ^a	25.73 ^b	11.21 ^{de}	0.0230	<0.0001
Maize+	20.56 ^c	14.33 ^d	12.21 ^{de}	10.43 ^e	0.0230	
Sorghum	31.98 ^a	16.53 ^b	14.70 ^c	12.79 ^d	0.041	<0.0001
Sorghum+	17.88 ^b	11.59 ^d	14.93 ^c	9.55 ^e	0.0414	

^{a-e} means between rows of the same grain with different superscripts differ ($P < 0.05$)

A time interaction was observed ($P < 0.0001$), showing that the highest impact of BioProtect™ occurred after 12 h of fermentation for both grains, with 19% and 18%-units decrease in Sd of BioProtect™ treated maize and sorghum, respectively. However, in ruminants, the extent to which starch is fermented in the rumen is also dependent on the retention time of the feed in the rumen, and that is more important when considering the proportion of rumen starch digestion. A 7 h is considered as a reasonable mean retention time of concentrate in the rumen of lactating dairy cows (Allen and Piantoni, 2014; Gallo et al., 2016). After 7 h of rumen retention time, starch not fermented in the rumen flows to the small intestine, where enzymatic starch digestion occurs and therefore not considered for the ruminal starch pool. In this study, the Sd for untreated grains reached 57% and 47% at 6 h in maize and sorghum, respectively, which was reduced to 45% and 37% in BioProtect™ treated maize and sorghum, respectively (Figure 5.3). At 6 h, BioProtect™ decreased the Sd by 12% and 10%-units in maize and sorghum, respectively. This result implies that more starch can possibly be shifted to the small intestines by means of BioProtect™.

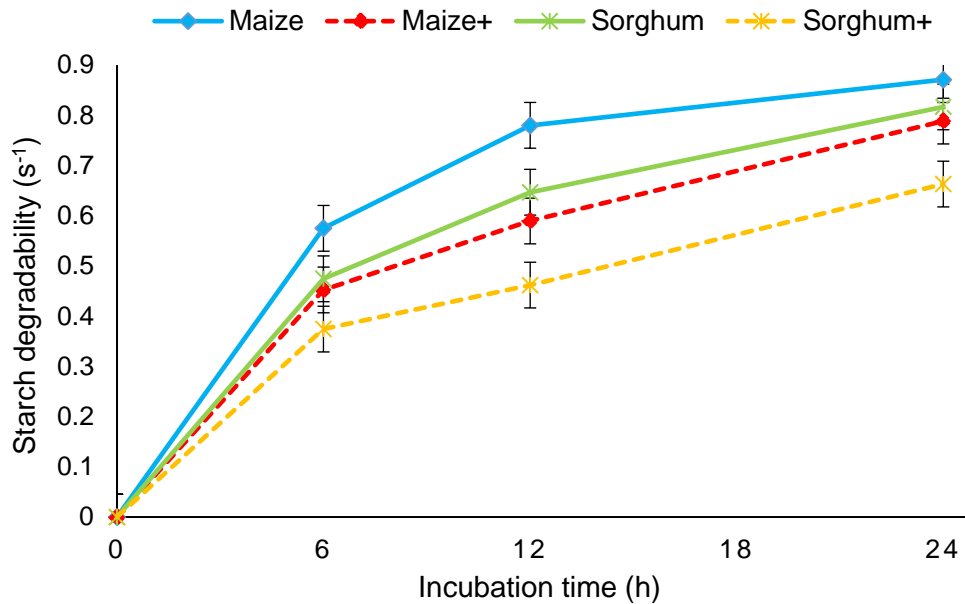


Figure 5. 2. Effect of BioProtect™ on maize and sorghum starch degradation at 0,6,12 and 24 h incubation.

Overall, untreated grains showed higher degradability throughout the incubation period, with most of the starch that disappeared by 24 h of incubation. This is contrast to that of Bioprotect™ treated grains. By 48 h, all combinations had reached a similar ($P > 0.1$) Sd value, showing how neither BioProtect™ nor particle size affected the extent of starch digestion. Figure 5.4 shows the effects of BioProtect™ on starch degradability, when pooling grains, across time and particle size. Overall, Bioprotect™ slowed the fermentation of starch for both grains.

Gonzalez et al. (2014) reported with *in vivo* trials that feeding sheep with rumen protected wheat by means of BioProtect™ (0.8%) that reduced rumen fermentation does not decrease whole tract digestibility of starch. This was presumably because there is adequate enzymatic starch digestion in the small intestine. Contrarily, Van Zyl, (2017) reported that BioProtect™ decreased the total tract starch digestibility (94.47% untreated vs. 91.47% treated maize) in dairy cows, suggesting that protected maize starch may not be available for digestion in the small intestine. Post-ruminal enzymatic digestion of starch may have been hampered or limited (Harmon et al., 2004), resulting in reduced total tract digestibility.

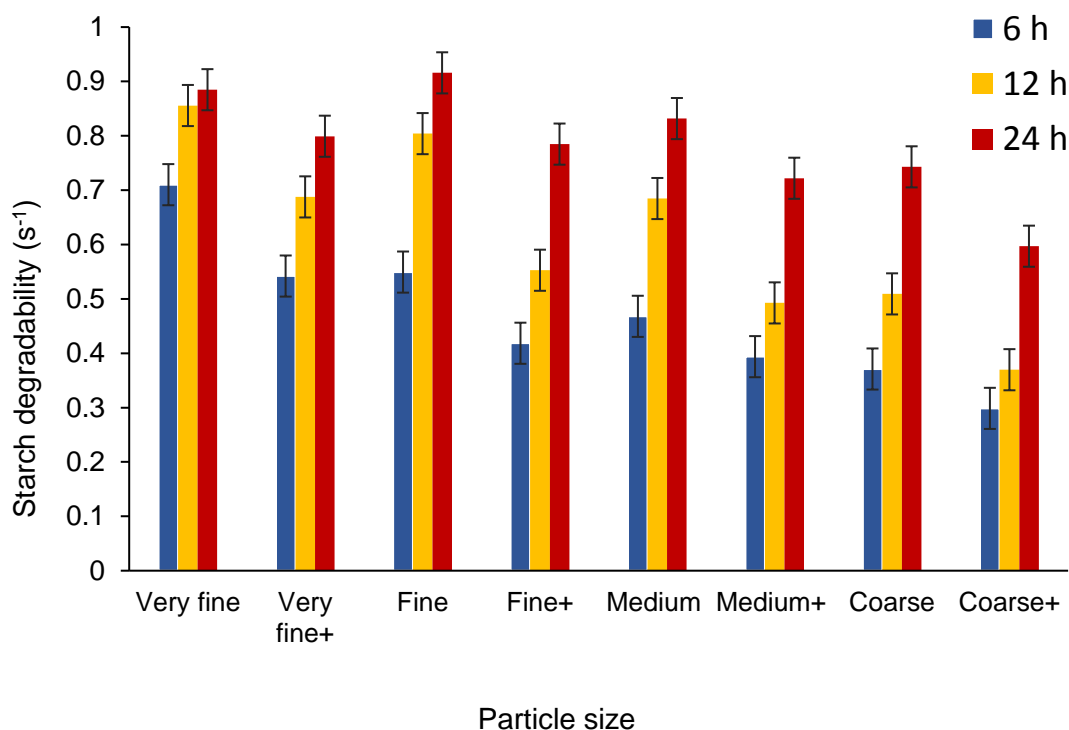


Figure 5. 3. Effect of BioProtect™ on starch degradability, when pooling grains, across time and particle size.

5.4.3. Simulation of the effects of BioProtect™ on starch digestibility in dairy cows

To evaluate the effect of BioProtect™ on starch digestibility, energy and microbial protein estimations, simulations were carried out using the NDS software (CNCPS version 6.5, RUM&N, Reggio Emilia, Italy). Sixteen dairy rations with either maize or sorghum, varying in particle size, treated or untreated (BioProtect™) were used. The standard composition of the TMR used in the simulations is shown in Table 5.4 and was formulated to contain 27% starch content using ingredients commonly used in South African dairy rations. The values of the starch, NDF and crude protein, and k_d for maize and sorghum of each simulation were replaced with the specific values obtained from the *in vitro* study. A third lactation high-yielding dairy cow weighing 650 kg, producing 40 kg of milk per day (3.5% fat and 3.3% protein) was used in the simulation. Dry matter intake was 27 kg/day. The same dairy cow and environment descriptions were used in all simulations. The effects of BioProtect™ on the predicted starch digestibility, metabolizable energy (ME) and metabolizable protein (MP) were evaluated.

Table 5. 4. A typical composition of the total mixed ration (TMR) used in the simulations.

TMR- Ingredients	% of DM	Nutrient Composition	% DM
Maize silage	35.69	DM, (%)	51.14
Lucerne hay	17.84	Crude protein	16.00
Maize grain*	19.36	Starch	27.15
Brewers grain wet	6.04	Neutral detergent fibre	32.56
Canola meal expelled	3.03	ADF	20.94
Extruded soybean meal	2.98	ADL	3.99
Citrus pulp dry	1.46	Non fibrous carbohydrates	38.59
Cottonseed fuzzy	5.29	Ether extract	5.34
Corn dist ethanol	4.38	Ash	7.70
Blood meal	0.74	Calcium	0.86
Megalac	0.48	Phosphorus	0.38
Mineral	2.71	Magnesium	0.34
		Potassium	1.25

*Maize grain fractions (very fine, fine, medium or coarse).

The simulated effects of BioProtect™ on starch digestibility, ME and MP of maize and sorghum varying in particle sizes are presented in Table 5.5. Overall, BioProtect™ reduced rumen starch fermentation, simulated increased rumen starch escape and increased post rumen starch digestibility for both grains, with effects more pronounced in maize grain based diets. These simulations concur with studies reporting that BioProtect™ can shift starch to the small intestines (Dunshea et al., 2012; Gonzalez et al., 2014). If more starch can pass through the rumen without being fermented it may reduce the incidence of rumen acidosis and may allow for greater post-ruminal enzymatic digestion of starch (Dunshea et al., 2012), given that it's digestion in the small intestine is not hampered or limited (Harmon et al., 2004; Reynolds, 2006). Table 5.6 shows the simulated amounts of starch digested throughout the gastrointestinal tract of dairy cows. The total tract digestibility of starch, according to the CNCPS 6.5, was comparable between BioProtect™ treated and untreated grains, suggesting that BioProtect™ did not decrease whole tract digestibility of starch, concurring with Gonzalez et al. (2014).

Table 5. 5. Simulated effects of BioProtect™ on starch digestibility, metabolizable energy and metabolizable protein of maize and sorghum varying in particle sizes.

	Starch digestibility				ME, Mcal/day	MP g/d
	Rumen fermentation (% of starch intake)	Rumen escape (%)	Post rumen digestion (% of entering)	Total tract digestibility (% of starch intake)		
Maize						
Very fine	78.5	21.5	18.0	96.46	66.99	3103.0
Very fine+	74.7	25.3	21.2	95.89	66.86	3033.9
Fine	78.3	21.7	18.1	96.43	66.90	3118.3
Fine+	71.0	29.0	24.3	95.35	66.67	2993.2
Medium	76.4	23.6	19.1	95.58	67.27	3133.4
Medium+	68.5	31.5	25.5	94.00	66.84	2999.9
Coarse	66.7	33.3	25.8	92.56	67.95	3059.1
Coarse+	65.9	34.1	26.5	92.35	67.88	3046.5
Sorghum						
Very fine	78.2	21.8	17.6	95.96	65.86	3067.3
Very fine+	73.4	26.6	21.6	94.99	65.62	2981.4
Fine	72.5	27.5	22.3	94.81	65.61	3012.2
Fine+	68.7	31.3	25.4	94.04	65.36	2950.0
Medium	71.5	28.5	22.3	93.82	64.74	2973.2
Medium+	71.7	28.3	22.2	93.85	64.77	2976.8
Coarse	69.9	30.1	22.6	92.54	64.74	2961.2
Coarse+	66.6	33.4	24.9	91.55	64.46	2913.8

Table 5. 6. Simulated values of rumen fermentation, post rumen digestion and total tract digestibility of starch in maize and sorghum varying in particle size.

	Starch digestibility (g)		
	Rumen fermentation	Post rumen digestion	Total tract digestibility
Maize			
Very fine	5806.7	1331.6	7138.3
Very fine+	5527.0	1569.3	7096.3
Fine	5774.2	1338.0	7112.2
Fine+	5240.2	1797.4	7037.6
Medium	5703.4	1428.7	7132.1
Medium+	5115.0	1899.3	7014.3
Coarse	5103.5	1974.3	7077.8
Coarse+	5037.7	2023.7	7061.4
Sorghum			
Very fine	5736.1	1298.2	7034.3
Very fine+	5382.7	1580.9	6963.6
Fine	5328.5	1635.3	6963.8
Fine+	5035.1	1859.3	6894.3
Medium	5226.8	1630.5	6857.3
Medium+	5242.5	1623.6	6866.1
Coarse	5132.7	1660.7	6793.4
Coarse+	4890.7	1830.5	6721.2

For all diets simulated, fine particles had the highest total tract digestibility (more than 95%) with most starch digested in the rumen (more than 70%) (Table 5.5), concurring with previous studies (Theurer, 1986; Huntington, 1997; Firkins et al., 2001) and meta-analysis by Ferraretto et al. (2013). The average simulated total tract starch digestibility of coarse particles was approximately 92% in both grains, which was lower than that of finer particles. In agreement with previous studies (Ferraretto et al., 2013), rumen starch escape increased with increasing particle size (Table 5.5). In comparison to coarse particles, rumen starch escape was 11.8 and 8.8% less in untreated and treated finer maize based diet, respectively, and 8.3 and 6.8 % less in untreated and treated finer sorghum-based diet, respectively.

The simulated ME supply was comparable between treated and untreated grains, averaged 67.17 and 65.15 Mcal/day in maize and sorghum-based diet, respectively (Table 5.5). This result implies that shifting starch to the small intestine may not increase ME supply. The effects on MP varied among diets but consistently showed lower simulated values with BioProtect™ treated grains (Table 5.5), possibly due to the

decreased amounts of starch digested in the rumen (Reynolds, 2006). It has been reported that increasing ruminal starch digestion can increase microbial protein synthesis in the rumen, hence shifting starch to the small intestine decreases the energy available for microbial protein synthesis.

Overall, the simulations confirmed that particle size was more effective in shifting starch digestion from the rumen to the small intestine than BioProtect™. While the use of the product results in statistically significant differences, as seen above, the use of processing alone might have a stronger biological effect.

5.6. Conclusion

The present study confirmed that rumen starch degradation is inversely related to increasing particle sizes. In particular, *in vitro* rumen starch degradability and rate of starch degradation decreased linearly with increasing particle size. Reducing the particle size increases the surface area available for microbial and enzymatic digestion, thereby enhancing the rate and extent of starch digestion. BioProtect™, a starch binding agent, reduced *in vitro* rumen starch degradation of both maize and sorghum grains, giving the opportunity of increasing the amount of starch shifted to the small intestine. The product was more effective with smaller than coarser particles, suggesting that it is more effective when starch is highly degradable. Simulations indicated how the use of BioProtect™ can reduce rumen starch digestibility, thereby increasing rumen starch escape and post rumen starch digestibility. The simulated total tract digestibility was not decreased by the use of BioProtect™, but MP was slightly reduced. Although the results of this study showed positive effects of BioProtect™ on reducing rumen starch degradation, in our simulations, larger particles were more effective at shifting site of digestion and it could therefore be a more cost-effective option, assuming a consistent total tract starch digestibility.

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CHAPTER 6

Effects of starch sources and particle size on digesta flow, starch digestibility, ruminal fermentation parameters and lactation performance of dairy cows

6.1. Abstract

The aim of the study was to intentionally shift site of digestion for starch. Specific objectives were to evaluate the effects of starch sources varying in particle sizes on digesta flow, starch digestibility, ruminal fermentation and performance of dairy cows. Four ruminally-cannulated multiparous Holstein cows (718 ± 59 kg of BW; 230 ± 57 DIM, 25.29 ± 6.80 milk/d) were used in a 4×4 Latin square design with a 2×2 factorial arrangement of treatments. Treatments were: the combinations of two starch sources (maize or sorghum, M or S) either finely or coarsely ground (using a 1- or 4-mm screen sieve, F or C) and fed within a total mixed ration. Diets were formulated to contain similar starch concentration. Digesta flow was quantified using the reticular sampling technique, applying the triple-marker method. Data were analyzed using a mixed model with cow as a random factor. The geometrical mean particle size of maize and sorghum milled at 1 or 4 mm were 255.6, 550.1, 250.6, and 728.5 μm , for MF, MC, SF, and SC, respectively. Starch concentration of the grains was 76.53, 73.99, 72.13 and 71.40% and *in vitro* 7 h starch degradability was 76.49, 63.51, 65.48 and 60.02%, for MF, MC, SF and SC, respectively. Dry matter intake was unaffected by dietary treatment, but cows fed maize diets consumed 3.21 kg/d more than cows fed sorghum diets (25.16 vs. 21.95 kg/d). Milk yield and composition were not affected by dietary treatments, however, MUN was lower for cows fed maize diets. Cows fed finer grains produced 1.46 and 2.86 kg/d more milk compared with cows fed coarser grains (maize and sorghum, respectively). Rumen and reticulum pH were greater for the SC diet. Propionate concentration was greater for both maize diets, however finely ground maize had lower acetate concentration, thereby decreasing the acetate:propionate ratio. Ruminal ammonia N was lower for the fine maize diet than the sorghum diets. Dietary treatments did not affect OM and NDF intake, nutrient flow of DM, OM and NDF, or ruminal digestibility of OM. Starch from the coarser maize was less ruminally digested (83.76 vs. 88.77.) and had a greater flow to the abomasum

when compared to the fine (1.04 vs 0.76). Total-tract starch digestion was increased in cows fed the MF diet compared with those fed coarse maize (96.29 vs. 87.84%). The NDF ruminal digestibility was lower in the MF diet compared to the other diets. Coarse maize tended therefore to be more digested post-ruinally. This study confirms that coarser particles can allow part of starch digestion to be shifted from the rumen to the small intestine. However, total starch digestibility could be decreased if ruminal digestion is not compensated for by small intestine or large intestinal digestion. The results of this study did not provide evidence to support the HOT, given that the higher propionate production in cows fed finer maize did not decrease feed intake.

6.2. Introduction

Starch, supplied mainly by cereal grains represents a substantial fraction of high-genetic merit dairy cow diets, ranging from <25 to >30% of dry matter (Patton et al., 2012; Allen and Piantoni, 2014) to meet their energy demands for high milk production by providing glucose and glucose precursors. The fate of starch in the rumen and post rumen of dairy cows is highly variable (≤ 30 to $\geq 90\%$ ruminal starch digestibility; Moharrery et al., 2014) depending on the interrelations among several intrinsic factors, primarily particle size, grain type and processing, and extrinsic factors which have been extensively discussed (Nocek and Tamminga, 1991; Huntington, 1997; Mills et al., 1999, 2017; Callison et al., 2001; Patton et al., 2012; Ferraretto et al., 2013; Giuberti et al., 2014; Owens et al., 2016). Moreover, factors such as particle size and gravity, buoyancy of particles in the rumen, level of fibre in the diet and level of feed intake may affect ruminal digesta flow (Allen, 1997; Firkins et al., 1998; Huhtanen et al., 2006) and ultimately total tract starch digestion.

Altering the dietary concentration and ruminal fermentability of starch has great effects on the performance of dairy cows by affecting the energy intake and partitioning, as well as absorbed protein (Allen, 2000; Allen et al., 2009). Moreover, according to the hepatic oxidation theory (HOT), highly fermentable starch reduces feed intake due to the increased production of propionate in the rumen (Allen et al., 2009). The hypophagic effects of propionate is more pronounced when cows are in a lipolytic state, with elevated

concentration of plasma non-esterified fatty acids (NEFA) from mobilization of body fat reserves (Allen et al., 2009; Stocks and Allen, 2012, 2013). During the transition period, feed intake is controlled predominately by the oxidation of fuels in the liver, whereas ruminal distension is a primary limitation of feed intake as lactation proceeds toward its peak (and as the lipolytic state diminishes). Propionate often decreases feed intake in late lactation (Allen, 2000; Allen et al., 2009). According to the HOT, the physiological state determines the effects of starch fermentability on DMI, production and reproductive responses (Bradford and Allen, 2004, 2007; Allen et al., 2009).

The concept of partially shifting the site of starch digestion has been proposed for the past years to overcome the negative effects associated with excessive rumen starch fermentability (Firkins et al., 2001; Larsen et al., 2009; Allen et al., 2009) and to benefit from its energetic efficiency when digested in the small intestines (Owens et al., 1998; Harmon and Mcleod, 2001; Huntington et al., 2006), to optimize starch utilization and feed efficiency. Although the site of starch digestion can be modulated by differing degrees of grain processing, chemical treatment or by feeding different grain sources, was previously studied (McAllister et al., 1990; Knowlton et al., 1998; San Emeterio et al., 2000; Beauchemin et al., 2001; Callison et al., 2001; Rémond et al., 2004; Taylor and Allen, 2005; Larsen et al., 2009; Mikula et al., 2011; Deckardt et al., 2013; Dunshea et al., 2013). Inconsistent results have been reported in the literature in terms of feed intake, production and reproduction response (Nocek and Tamminga, 1991; Reynolds, 2006). There is paucity of data on the proportion of starch fermented in the rumen, flowing to and digested in the small intestine. Moreover, the optimal site of starch digestion is still unclear, reinforcing the need for further investigation to better understand the effects of this concept of shifting the site of starch digestion.

The objective of this study was to evaluate the effects of starch sources and particle sizes on nutrient flow, starch digestibility, ruminal fermentation parameters and lactation performance of dairy cows. The hepatic oxidation theory was also investigated in late lactation. It was hypothesized that feeding coarsely ground maize and sorghum grains would increase the flow of starch to the small intestine and consequently minimize the negative effects associated with excessive rumen starch digestibility compared to finely ground grains. This study will generate needed information about the starch flow

and ruminal and post ruminal starch digestion, for use in modelling and in the formulation of more efficient diets in order to optimize the utilization of starch and productivity and ultimately cow profitability. Moreover, understanding the HOT may help us understand the response of dairy cows to diets, to better formulate diets to optimize feed intake, increase production, improve nutrition, health and better welfare of dairy cows.

6.3 Materials and Methods

All experimental procedures were approved by the Research Ethics Committee: Animal Care and Use of Stellenbosch University, South Africa (protocol number: SU-ACUD15-00064). The experiment was conducted at the Welgevallen Experimental Farm of Stellenbosch University and carried out in accordance with the South African National Standard for the care and use of Animals for Scientific Purpose (SANS 10386: 2008).

6.3.1 Animals, experimental design and diets

Four multiparous lactating Holstein-Friesian dairy cows (718 ± 59 kg of BW; 230 ± 57 DIM, 25.29 ± 6.80 MY/d at the beginning of the trial; values expressed as means \pm SD) fitted with ruminal cannulas (10 cm i.d.; Bar Diamond Inc., Parma, ID) were used in a 4 x 4 Latin square design experiment. The experiment consisted of four different diets which were evaluated on 4 different lactating dairy cows during 4 periods. Each experimental period lasted for 16 d, with the first 10 d for adaptation and the last 6 d used for data collection. Owing to the small differences between treatments, adaptation periods were shorter compared to what is usually suggested (Grant et al., 2015). Recently, Grant and collaborators (2015) demonstrated that response to diet for eating, ruminating and resting behaviour stabilizes within 1 to 7 days, therefore, an adaptation period of 7 to 14 days is usually adequate for experiments investigating DMI, performance and eating behaviour, except for diets with extreme differences in their level of digestibility. We aimed to reduce the adaptation periods in order to minimize confounding effects due to milk yield reduction and physiological changes occurring towards the end of the lactation. Cows were individually housed in roofed pens bedded with sand and with continuous access to water throughout the experiment. The study was conducted between December 2016

and February 2017 with environmental temperature ranging from 15 to 34°C, with an average of 21°C.

This study consisted of four experimental diets made up of two different cereal grains (maize or sorghum) either finely ground or coarsely ground. Maize (*Zea mays*) and sorghum (*Sorghum bicolor*) were sourced locally. Cereal grains were milled using a hammer mill (Drostsky, model F 16 A). To produce the fine and coarse treatments, the hammer mill was fitted with a 1-mm or a 4-mm screen representing fine and coarse particle sizes, respectively. The size of the sieves used in this experiment was selected based on results of the preliminary *in vitro* study presented in Chapter 3 (Titled: *Invitro* starch digestibility and rate of starch degradation of different particle sizes of cereal grains commonly fed to dairy cows).

Prior to the experiment, the particle size distributions (% of DM retained on each sieve) and geometric distribution of particle sizes (GMPS) of milled maize and sorghum grains was determined by sieving. The GMPS is the most common method used to compare particle size distribution of cereal grains (ASABE, 2007). A representative sample (~100 g) was sieved for 20 minutes through a series of 7 screen sieves with nominal aperture sizes of 125, 250, 500, 850, 1180, 2000 and 3350 µm using a sieve shaker (Kingtest laboratory test sieve, Retsch GmbH, Series AS 200 basic, Germany) at an amplitude of 100. A bottom pan was included to retain particles less than 125 µm. Each sieve was individually weighed before and after each sieving to obtain the weight of the samples on each sieve, hence determining particle size distribution and the geometric mean particle size. Three runs per cereal grain were done, and sieves were cleaned thoroughly with a brush per run. No particles were retained in the 3350 µm sieve for both grains. The mean particle size was calculated using a log normal distribution (Baker and Herrman, 2002). The nominal geometric mean particle size (NGMPS) was calculated, which is based on the smallest dimension of square openings in sieves. Besides the particle size distribution, milled grains (1 and 4 mm; maize and sorghum) were also fermented for 7 h to determine the *in vitro* starch degradability according to the method of Goering & Van Soest. (1970) as described in Chapter 3. Rates of starch degradation were then computed using the 7-h formula (Sniffen and Ward, 2011) as follows:

$$K_d-7 = (((LN(\text{Starch, \%DM})) - (LN(((100 - 7h \text{ ivSd, \%Starch}) / 100) * \text{Starch, \%DM}))) / 7) * 100.$$

where starch% is the starch content of the grain and ivSd% is the *in vitro* starch degradability as % of starch.

Experimental diets were fed as a total mixed ration (TMR) containing (DM basis) 37.7% starch source (maize or sorghum; fine or coarsely ground), 41.5% Lucerne, 1.9% wheat straw, and 18.9% high protein concentrate (Afgri HPC; Afgri Animal Feeds, Centurion, GP, South Africa). Lucerne was chopped together with wheat straw using a feed mixer (Storti Husky MT 50) to obtain a theoretical chop length of 4cm. The TMR was formulated to meet the dietary requirement of lactating dairy cows according to National Research Council (NRC, 2001) guidelines. Experimental diet ingredients (Lucerne+ wheat straw, milled grains and HPC) were weighed and mixed in a concrete mixer per feeding time per animal, with addition of water (~ 48 to 50% moisture content). Cows were fed twice daily (equal portions) at 07:30 and 17:00, after each milking which was done at 06:00 and 16:00. The sequence of the treatments was balanced to handle carry over effects. The ingredient and chemical composition of the experimental diets are presented in Table 6.1.

Table 6. 1. Ingredients and chemical composition of the experimental diets fed as TMR to lactating dairy cows.

	Dietary treatments			
	Maize		Sorghum	
	Fine-ground	Coarse-ground	Fine- ground	Coarse-ground
Ingredients, % of DM				
Lucerne hay	41.5	41.5	41.5	41.5
Wheat straw	1.9	1.9	1.9	1.9
Maize (yellow)	37.7	37.7	—	—
Sorghum (red)	—	—	37.7	37.7
Commercial HPC ¹	18.9	18.9	18.9	18.9
Nutrient Composition ² , % of DM				
DM, % as fed	50.72	50.27	50.60	50.22
OM	92.98	92.95	92.88	92.78
Ash	7.02	7.05	7.12	7.22
Starch	26.34	26.22	25.03	24.66
CP	17.27	17.41	18.91	19.23
EE	2.59	2.67	2.69	2.59
NDF	31.47	32.06	30.77	31.51
iNDF	16.21	15.96	16.04	16.32
ADL	4.27	4.34	4.31	4.36
pdNDF ³	15.26	16.10	14.74	15.19
NFC ⁴	41.66	40.82	40.51	39.45

¹HPC (high protein concentrate) was sourced from Afgri (Afgri Animal Feeds, Centurion, GP, South Africa), contained Amminomax soya, Gluten 21, Blood meal spray dried, Molasses, Urea, Limestone, Salt, Premix, Poultry by-product, Monocalcium Phosphate.

²DM=Dry matter; OM=Organic matter; CP=Crude protein; EE=Ether extract; NDF=Neutral detergent fibre; iNDF=indigestible NDF; ADL=Acid detergent lignin; pdNDF=potentially digestible; NFC=Non-fibrous carbohydrates.

³Calculated as NDF – iNDF.

⁴Calculated as 100 – (CP % + NDF % + ether extract % + ash %; NRC, 2001).

6.3.2 Preparation and infusions of markers

The digesta flow from the rumen was quantified using reticular sampling (Krizsan et al., 2010) techniques, with modification and applying the triple-marker method (France and Siddons, 1986). The three markers used were: Cobalt ethylenediamine tetra-acetic acid (Co-EDTA), associated with the fluid/liquid phase, Ytterbium-acetate (Yb-acetate) for the small/solid particle phase and indigestible neutral-detergent fiber (iNDF) for the large particle phase. The Co-EDTA was prepared as described by Udén et al. (1980) and Yb-acetate was obtained from a commercial source (Sigma-Aldrich, Gauteng, South Africa). The indigestible NDF that is naturally present in the diet was used as an internal marker.

Markers (Co-EDTA (~18 g/d) and Yb-acetate (~3.78 g/d) were dissolved in 3 L of distilled water per cow per day during each period. Twenty litres of markers were prepared per cow per period. After dissolving and mixing markers for individual cows, markers were transferred into a 100 L plastic drum and well-mixed to get a homogenous marker solution. A sample of marker solution was collected to determine the actual concentration of markers.

The infusion of markers (Co-EDTA (2.5 g/d of Co) and Yb-acetate (1.5 g/d of Yb) started on d 11 in each experimental period until the last sampling of digesta on d 16, and reticular sampling was carried out on the last 3 d of the experimental period (day 14-16). Each cow received the marker solution (CoEDTA and Yb) directly into the rumen at the rate of 125 ml/h via cannula fitted with the infusion line (i.d. = 4 mm), using an injector (Simcro™, New Zealand). On the first day of infusion (d 11), a prime dose of 1.5 times the daily infusion was given prior to the hourly infusion, used to reach a rapid equilibrium of the ruminal marker concentrations.

6.3.3 Measurements and sampling procedures

6.3.3.1 Feed intake and feed and ort samples: To determine the feed intake, feed offered, and orts were recorded once daily per cow before morning feeding for the entire experiment. However, only data from d 11 to d 16 were used for data analysis. The amounts of TMR offered were adjusted daily throughout the trial depending on the

previous day's intakes to allow about 5-10% oforts. Samples of TMR and orts were collected daily from d 11 to d 16, stored at -20°C until further analysis.

6.3.3.2 Reticular digesta sampling: Using the reticular sampling technique, 400-mL reticular samples were collected over 3 consecutive days, four times daily at the interval of 6 h. The sampling times were 08:00; 14:00; 20:00; 02:00 on d1, 10:00; 16:00; 22:00; 04:00 on d2 and 12:00; 18:00; 00:00 and 06:00 on d3 of sampling, allowing 12 representative samples to be collected. On the last 2 days of sampling, the sampling occasion were moved 1 h later than on the previous day so that the sampling was equally distributed to represent a 24 h feeding cycle. Briefly, reticular digesta was sampled according to Krizsan et al. (2010), with modification. In this study, a Selekt Cattle Pump and Rumen Fluid Collector (Nimrod Veterinary Products Ltd., Moreton-in-Marsh, Gloucestershire, UK) was used to collect reticular digesta. The Selekt Cattle Pump and Rumen Fluid Collector was inserted into the reticulum through the rumen while holding the steel tip of the pump, which has holes to prevent rumen digesta from flowing into the pump. While in the reticulum the tip of the pump was released, flushed about 3 to 4 times before collecting 1.0 L of reticular digesta. The reticular digesta pH was measured immediately after sampling using a portable pH meter (Crison PH25 pH meter, Lasec, SA). The 1.0 L of reticular sample of digesta was divided into subsamples as follow: 400 ml reticular digesta for digesta flow analysis, 250 ml for microbial analysis and 150 ml for spare. Samples were kept in crushed ice immediately after collection. The 400 ml reticular subsamples from every time point were pooled and held at -20°C as they were collected to yield a 4.8L reticular composite samples from each cow in each period. It is important to mention that the reticular digesta samples were not filtered/sieved to discard large particles as described by Krizsan et al. (2010). Beside the modifications in the procedure for reticular digesta sampling, Krizsan et al. (2010) reported that sieving of reticular samples below 1 mm does not give representative samples of that are likely to leave the reticulum and it seems like the triple-marker method was not able to completely correct for this unrepresentative sampling. Moreover, Fatehi et al. (2015) reported that primary sieving of ruminal and reticular digesta through an 11.6-mm sieve did not differ from faecal particle size distribution, implying that digesta particles smaller than this were eligible to flow out of the rumen.

6.3.3.3 Rumen fluid and faecal sampling: Rumen fluid samples were collected from the ventral area of the rumen before the reticular sampling and rumen pH was measured immediately as described in reticular digesta sampling. The rumen fluid samples were placed in crushed ice immediately after sampling and stored at -20°C pending VFA and ammonia (NH₃) analyses. Faecal samples were quantitatively collected (approximately 250 g) on the same 3 d as the reticular and rumen sampling per cow, applying the similar sampling time schedule. Faecal samples were collected from the rectum or off ground on a concrete floor immediately after defecation, and not mixed with urine. Samples were stored at -20°C pending further processing. Faecal samples were used to determine the total tract digestibility using iNDF as internal marker and for marker recoveries.

6.3.3.4 Milk yield and samples; and body weight: Cows were milked twice daily at 06:00 and 16:00, and milk yield was recorded at each milking automatically using the AfiMilk dairy farming system (AfiMilk Ltd, Kibbutz Afikim, Israel) throughout the experiment. Milk samples were taken from morning and afternoon milkings for four consecutive days starting from d 13 to d 16 of each period. Daily milk samples (~40 ml) composites were obtained by pooling morning and afternoon milk samples based on the proportion of milk yield per cow at each milking. Composite milk samples were preserved with broad spectrum microtabs, stored at room temperature and delivered to a SANAS accredited testing laboratory, MilkoLab (GE Dairy Supplies, Parow, Cape Town, South Africa) for milk components analysis. Body weight (BW) was recorded daily during milking throughout the experiment using the AfiWeigh system (AfiMilk Ltd, Kibbutz Afikim, Israel). However only weight recorded from three consecutive days at the beginning and the end of each period was used to calculate the mean BW of cows for each experimental period.

6.3.4 Preparation of samples

6.3.4.1 Reticular digesta samples: After each experimental period, 4.8 L reticular digesta samples, which were pooled by period for each cow were thawed at room temperature and separated into three different phases: reticular large particle (LP), reticular small particle (SP), and reticular fluid (liquid) phase (FP) as described by Reynal

and Broderick (2005), with modifications. Briefly, reticular samples were squeezed through 1 layer of cheesecloth and particles retained on the cheesecloth were defined as the large particle phase. The filtrate was transferred to a 250-ml centrifuge bottle (Nalgene, model 2189-0008, Thermo Fisher Scientific, Waltham, USA) and centrifuged at 14,000 rpm (rotor, J14) for 5 min at 4°C using a Beckman coulter centrifuge (Avanti j-e series, Beckman Coulter, USA). The supernatant was poured off and defined as the fluid phase and the pellet was defined as the small particle phase. The separated phases were stored at -20°C until lyophilized. The large particle phase was ground to pass through a 1-mm sieve using a Wiley mill (Model 4, Thomas-Scientific, USA), and the small particle and liquid phase was ground using a coffee grinder prior analysis. The concentration of Co, Yb, and iNDF determined in the LP and SP and of Co and Yb determined in the FP were used to mix DM from freeze-dried FP, SP, and LP in the correct proportions to reconstitute the reticular true digesta (RTD) flowing out of the rumen based on the triple-marker method of France and Siddons (1986). Concentrations of Co, Yb, and iNDF were greater than the other markers in FP, SP and LP, respectively, allowing for application of the triple-marker approach. The RTD were analysed for DM, ash, starch, CP, EE, NDF, ADF, and iNDF.

6.3.4.2 Feed, orts, faecal samples and rumen samples: Feed, orts and faecal samples were thawed at room temperature, pooled per cow over each sampling period, thoroughly mixed by hands and subsampled. Samples were dried in a forced air oven at 60°C for 72 h to determine DM content and ground to pass through a 1-mm sieve using a Wiley mill (Model 4, Thomas-Scientific, USA) pending analysis. Feed, orts and faecal samples were analysed for DM, ash, starch, CP, NDF, ADL and iNDF. Rumen fluid were thawed at room temperature and subsamples were taken for VFA and NH₃ analysis per sampling time.

6.3.5 Indigestible NDF and Chemical analyses

The concentration of iNDF in all TMR samples, digesta fractions (LP, SP, but not the fluid phase; Ahvenjärvi et al., 2000), orts and faecal samples was determined using a long term (240 h) *in-vitro* fermentation as described by Raffrenato et al. (2018). Concentrations of external markers (Cobalt [Co] and Ytterbium [Yb]) in the individual

digesta phases, faecal samples and marker samples were analysed using inductively coupled plasma Optical Emission Spectroscopy (ICPOES/AES) (Thermo iCAP 6000, Thermo Scientific). Briefly, samples (± 0.3 g) were weighed directly into the Microwave digester Teflon vessels. Nitric acid (HNO_3) (3.5 ml) and hydrogen peroxide H_2O_2 (1 ml) were added to predigest samples for about 15 min before adding deionized water (2.5 ml). Samples were digested using CEM MARS microwave digester at 210 °C and 800 PSI. Vessels were cooled, and samples were diluted (x10) to reduce the acid concentration prior to analysis for Co and Yb.

Milk samples were analysed for fat, protein, lactose, somatic cell counts (SCC) and milk urea nitrogen (MUN) by means of infrared analysis (CombiFossTM FT+, Hillerød, Denmark) at MilkoLab (GE Dairy Supplies, Parow, Cape Town, South Africa). Rumen volatile fatty acid (VFA) concentrations were determined using a gas chromatograph-mass spectrometry (GC-MS) (Thermo Scientific TriPlus RSHTM, TRACETM 1300, Milan, Italy), with clean-up procedure of rumen fluid samples (modified from Siegfried et al., 1984) which deproteinises the rumen fluid samples and removes the sugars, and using Crotonic acid as the internal standard. The concentrations of ammonia in the rumen fluid was analysed using NH_3 slides on the IDEXX VetTest Chemistry Analyser (IDEXX Laboratories PTY Ltd., Cape Town, South Africa), using a dilution factor of 20.

The DM and ash content were determined according to the AOAC (2002), official Method 934.01 (100°C; 24h) and 942.05 (500°C; 6h), respectively. The starch content was determined as described by Hall (2009). Crude protein (CP) was determined by Dumas combustion method according to the AOAC, official method 992.15 using a LECO nitrogen analyser (model FP-528, Leco Corporation, St. Joseph, MI, USA) and ether extract (EE) by using a Tecator Soxtec System HT 1043 Extraction Unit (AOAC, 2002; Method 920.39). Neutral detergent fiber (NDF) was analysed as described by Mertens (2002) using sodium sulfite and thermostable α -amylase, and acid detergent lignin (ADL) was determined according to Goering and Van Soest. (1970) as modified by Raffrenato and Van Amburgh (2011). Organic matter (OM) was calculated as $100 - \%\text{ash}$; Non-fiber carbohydrates (NFC) was calculated as $100 - (\%\text{CP} + \%\text{NDF} + \%\text{EE} + \%\text{ash})$ according to the NRC (2001); and potentially digestible NDF (pdNDF) was calculated as $\text{NDF} - \text{iNDF}$. The indigestible NDF (iNDF) in all TMR, orts and faecal samples was determined

by long term (240 h) *in vitro* fermentation as described by Raffrenato et al. (2018) and was used as an internal marker to estimate faecal excretion and apparent total tract nutrient digestibility. All samples were analysed in duplicate.

6.3.6 Calculations and statistical analysis

Nutrient intake was calculated using the amounts and compositions of feed offered and refused. Nutrient flow was calculated using the reconstitution system based on the 3 markers (co-EDTA, yb and iNDF). Total-tract nutrient digestibilities were calculated from iNDF as an internal marker and nutrient concentration in the refusals-adjusted diet and faeces as described by Ferraretto et al. (2015) using the following equation: Apparent total tract nutrient digestibility (% of nutrient intake) = $100 - [(TMR\ iNDF / faecal\ iNDF) \times (faecal\ nutrient\ content / TMR\ nutrient\ content)]$.

Particle size distributions and nominal geometric mean particle size (NGMPS) were analysed as response variables by the GLM procedure of SAS (version 9.3, SAS Institute, Cary – NC, USA), using a 2x2x6 factorial arrangement of grain type, screen and sieve size for particle distribution and NGMPS. All the respective interactions and run as random factor were also included.

Production data, nutrient flow and digestibility were analysed using the GLIMMIX procedure of SAS (Version 9.3; SAS Institute Inc., Cary, NC, USA) for a 4 x 4 Latin square design, with period and treatment as fixed factors and cow as random factor. Differences between treatments were determined by the least significant difference method with a Tukey adjustment. Rumen fermentation parameters (pH, ammonia and volatile fatty acids) data were analysed using the GLIMMIX procedure of SAS (Version 9.3; SAS Institute Inc., Cary, NC, USA) with time as a repeated measure. The model included the fixed effects of time, day, period, diet and the interaction between time and diet. Statistical differences were declared significant at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$. Results are reported as least square means (LSM) and respective standard errors (SEM).

6.4 Results and Discussion

6.4.1 Particle distribution and NGMPS of milled maize and sorghum grains

The particle size distribution and nominal geometric mean particle size (NGMPS) of maize and sorghum grains milled using a 1- or 4-mm screen sieve are presented in Table 6.2. The distribution of particles among the different sieves varied between grains and screen sizes. At 1-mm screen size most particles (80.19 and 80.24% of maize and sorghum, respectively) were retained on the $\geq 250 \mu\text{m}$ sieves, with little material (19.82 and 19.73%, respectively) on the $\geq 500 \mu\text{m}$ sieves. In contrast, when the hammer mill screen size was increased to 4-mm, the majority of the particles were retained on the $\geq 500 \mu\text{m}$ sieves (60.23 for maize and 76.23% for sorghum), with only 39.76 and 23.78% retained on the sieves lower than $250 \mu\text{m}$ for maize and sorghum, respectively. Generally, there was more material retained on the larger sieves ($>500 \mu\text{m}$) in sorghum milled at 4 mm compared to maize, which had relatively more on smaller sieves ($<125 \mu\text{m}$), indicating differences in inherent characteristics of the grains. Generally, the proportion of particles retained on the smaller sieves ($<500 \mu\text{m}$) increased with extent of processing of both grains, indicating greater breakage of grains with increased degree of milling (1-mm screen sieve). An interaction was observed between grain, screen and sieves ($P=0.0005$).

The NGMPS varied greatly, with the highest (728.54 ± 2.29) observed in sorghum milled at 4 mm, followed by maize at 4 mm (550.14 ± 2.56) and the lowest (250.62 ± 2.29 and 255.55 ± 2.56) reported in sorghum and maize at the 1 mm screen size, respectively. Reducing hammer mill screen size decreased the NGMPS for both grains, with effects more pronounced in sorghum (728.54 ± 2.29 vs. 250.62 ± 2.29). The interaction between the grain and screen size was significant ($P < 0.0001$). These results confirm that different mill screen sizes and grains result in different particle size distributions and geometric mean particle size (GMPS), which can influence starch digestibility (Ferraretto et al., 2013; Dias Junior et al., 2016; Gallo et al., 2016).

Table 6. 2. Particle size distribution and nominal geometric mean particle size (NGMPS) of maize and sorghum milled at 1 mm and 4 mm screen.

Sieves, ¹ (µm)	1 mm		4mm		P-value
	Maize	Sorghum	Maize	Sorghum	
2000	-	-	4.49	3.01	
1180	0.160 ^b	0.28 ^b	24.64 ^a	29.68 ^a	0.0005
850	0.687 ^c	0.98 ^c	16.21 ^b	25.68 ^a	0.0005
500	18.97 ^a	18.47 ^a	14.89 ^b	17.86 ^a	0.0005
250	34.21 ^a	32.97 ^a	14.67 ^b	10.94 ^b	0.0005
125	32.02 ^a	31.77 ^a	14.99 ^b	6.39 ^c	0.0005
pan	13.96 ^{ab}	15.50 ^a	10.11 ^{bc}	6.45 ^c	0.0005
NGMPS ² , µm	255.55 ^c	250.62 ^c	550.14 ^b	728.54 ^a	<0.0001

^{a-c}Means within a row with different superscripts differ (P<0.05).

¹Particle size distribution is given as the proportion (%) of total material retained on each sieve.

²NGMPS = Nominal geometric mean particle size.

- No material was retained on the 1 mm screen.

6.4.2 Nutrient composition and starch degradability of milled maize and sorghum grains

The chemical composition, starch degradability (Sd) and rate of starch degradation (k_d) of maize and sorghum grains milled at 1- and 4-mm screen are presented in Table 6.3. Overall, the composition of maize and sorghum were within the expected ranges (Huntington, 1997; NRC, 2001), with higher starch content in maize than sorghum, which averaged 75.26 and 71.76%, respectively. The starch concentration of MF and SF averaged 76.53% and 72.13, respectively and was 2.54 and 1.86 percentage units numerically greater than that of MC and SC, respectively. The higher starch content in finely ground grains than in coarsely ground grains could be attributed to increased surface area for enzymatic action by *alpha*-amylase, i.e. higher degree of access to starch (Al-Rabadi et al., 2009). The content of CP was similar between finely and coarsely ground grains because grains were finely ground to 1 mm with a Wiley mill before nitrogen analysis using a LECO nitrogen analyser. The Sd (k_d) were 76.49 (20.97), 63.51 (14.41), 65.48 (15.26) and 60.02 % (13.11 h⁻¹) for MF, MC, SF and SC, respectively. These results confirm that decreasing the mean particle size increases starch digestibility (Ferraretto et al., 2013) by increasing the surface area for bacterial and enzymatic digestion (Huntington, 1997).

The degradability of starch in sorghum was lower and slower than that of maize which could be related to their starch-protein matrix, which is more resistant to moisture, microbial attack and enzyme penetration (Rooney and Pflugfelder, 1986; Theurer, 1986; Herrera-Saldana et al., 1990; Kotarski et al., 1992; McAllister et al., 1993; Al-Rabadi et al., 2009, 2012) as well as by the presence of anti-nutritional factors that may bind to amylase, reducing its activity (Björck and Nyman, 1987).

Table 6. 3. Nutrient composition, degradability and rate of starch degradation of maize and sorghum milled with 1- and 4-mm screen.

	1 mm		4mm	
	Maize	Sorghum	Maize	Sorghum
Nutrient composition ¹ , % DM				
DM, % of fresh matter	86.51	87.43	86.84	86.67
OM	99.01	98.95	99.22	98.92
Starch	76.53	72.13	73.99	71.40
CP	7.73	12.90	7.73	12.90
Ash	0.99	1.05	0.78	1.08
EE	4.00	3.69	4.00	3.69
NDF	18.69	9.71	18.41	15.81
ADF	2.63	4.35	4.20	6.94
Starch digestibility, 7 h, ² %	78,14	68,30	63,51	60,06
k _d , ³ % h ⁻¹	22,52	14,41	16,84	13,11

¹DM=Dry matter; OM=Organic matter; CP=Crude protein; EE=Ether extract; NDF=Neutral detergent fibre; iNDF-indigestible NDF; ADF-Acid detergent fiber.

²Starch degradability measured *in vitro* after 7-h incubation.

³k_d = rate of starch degradation.

6.4.3 Feed intake, milk yield and composition

The dietary treatment effects on dry matter intake (DMI), milk yield and composition are presented in Table 6.4. The DMI was not affected by dietary treatments ($P = 0.1558$). The average DMI was 25.50, 24.81, 22.58 and 21.39 kg/d for MF, MC, SF and SC diets, respectively. Tendencies were observed between cows fed MF and SC as well as for MC and SC diet ($P = 0.0920$). The result of the current study are inconsistent with our expectations given the hepatic oxidation theory of the control of feed intake (Allen et al., 2009). Decreasing particle size by mechanical processing (grinding) increases

digestibility of starch in the rumen, and according to the HOT, highly fermentable starch increases the production of propionate which suppresses feed intake. The literature is inconsistent regarding the effects of particle size of grains on DMI. Recently, Brossillon et al. (2018) found that DMI was not affected by particle size, when mean particle size was 580 and 2,047 μm . Fredin et al. (2015) compared diets containing ground maize with mean particle size of 552 and 1,270 μm for fine and coarse, respectively and reported that DMI was not affected by particle size. Similarly, other studies (Knowlton et al., 1996, 1998; Callison et al., 2001; Rémond et al., 2004) have found that DMI was not affected by differing maize particle size, in agreement with a recent meta-analysis of 102 peer-reviewed journals by Ferraretto et al. (2013). Contrary, Yu et al. (1998) compared the effects of grinding on DMI (mean particle size was 1,180 μm for fine and 2,420 μm for coarse) and found that intake decreased for cows fed diets containing finely ground maize compared to cows fed coarsely ground maize, in agreement with a meta-analysis of 48 published papers by Firkins et al. (2001). Firkins et al. (2001) reported that DMI decreased with fine grinding compared to coarsely cracked maize. However, Yu et al. (1998) explained that the decreased DMI by cows fed finely ground maize was possibly due to increased dustiness. On the other hand, Knowlton et al. (1998, experiment 2) found that DMI increased for cows fed a diet containing fine maize when the mean particle size was 618 vs. 1,725 μm , contrary to their Latin square studies (experiment 1) where the DMI was unaffected by maize particle size.

Inconsistent results on the effects of particle size on DMI between studies could be attributed to differences in the mean particle sizes and to differences in the composition of experimental diets such as the dietary NDF content. It is well documented that the DMI is influenced by several dietary factors, with dietary NDF content being the major factor affecting DMI in ruminants, by rumen fill (Mertens, 1987). The NDF content of the diets ranged from 30.8 to 32.1%, which may have been less than required to elicit a negative response on voluntary DMI due to ruminal distension (Allen, 2000). Fredin et al. (2015) also observed similar results when the NDF content of the diets ranged from 26.9 to 32.1%. Moreover, the effects of diet on feed intake vary with the physiological state of the cow, with rumen fill limiting intake as lactation proceeds toward its peak (as milk yield increases) and by propionate during late lactation as the demand for glucose for milk

production decreases, resulting in greater propionate oxidation in the liver, suppressing feed intake (Allen, 2000; Allen et al., 2009). Cows used in this study were in the late stage of lactation, with more than 300 DIM at the end of the experiment, and with low milk yield, implying that feed intake would be mainly controlled by the increased production of propionate from finer particles. This was however not observed in the current study. The average dietary NDF content of our diets were within the levels recommended by NRC (~28-33%) for the maintenance of proper ruminal function (NRC, 2001).

Milk yield ranged from 22.51 to 25.37 kg/d, with numerically higher milk yield in cows fed fine grains than coarser grains. Although milk yield was not affected ($P > 0.05$) by dietary treatments, SC tended to result in lower milk yield ($P=0.06$) than SF and MF (2.86 and 2.77 kg/d, respectively). Controversy exists in the literature with regards to the effects of particle size on milk yield. Some studies reported increased (Knowlton et al., 1996, 1998; Rémond et al., 2004) or unaffected (Knowlton et al., 1998; Yu et al., 1998; San Emeterio et al., 2000; Callison et al., 2001; Fredin et al., 2015; Brossillon et al., 2018) milk yield for cows fed diets containing fine ground maize compared with coarse ground maize. In a meta-analysis, Ferraretto et al. (2013) reported that milk yield is unaffected by mean particle size, contrary to a meta-analysis by Firkins et al. (2001), who reported that fine grinding increases milk production as compared to coarser grinding. Higher milk yield in fine ground grain as compared to coarse ground grain diets could be attributed to greater ruminal starch digestibility, which increases the production of VFA and ruminal bacterial yields, supporting milk synthesis (Yu et al., 1998).

Milk composition (fat, protein and lactose) was not affected ($P > 0.05$) by dietary treatments. Milk fat and protein content have been reported to be unaffected by particle size in several studies (Knowlton et al., 1998; San Emeterio et al., 2000; Callison et al., 2001; Fredin et al., 2015; Brossillon et al., 2018) and in a meta-analysis by Ferraretto et al. (2013), which is contrary to Firkins et al. (2001) meta-analysis, which reported greater milk fat and protein content for coarse ground dry maize. Rémond et al. (2004) reported a tendency of increased milk protein content in fine ground maize as compared to coarsely ground maize. Yu et al. (1998) observed lower milk fat content for cows fed the diet containing finer grains compared to coarse grains, although not significantly different. According to the literature, ruminal starch digestibility is highly correlated with milk protein

percentage, but negatively correlated to milk fat percentage (Theurer et al., 1999; Firkins et al., 2001). Increased rumen starch fermentation is known to increase microbial protein synthesis, which is associated with greater protein synthesis in the mammary gland, thus, increasing milk protein (Theurer et al., 1999; Firkins et al., 2001; Jenkins and McGuire, 2006; Rius et al., 2010). Conversely, increased ruminal fermentable starch increases ruminal propionate, which increases gluconeogenesis, which in turn, stimulates insulin secretion, reducing fatty acid release from adipose tissue to the mammary gland, thus decreasing milk fat production (Bauman and Griinari, 2003). Moreover, with increasing ruminal starch digestibility milk fat content decreases as the ratio of acetate to propionate decreases, with increasing ruminal starch digestibility (Bauman and Griinari, 2001).

Milk urea nitrogen (MUN) concentration was affected by dietary treatment, lower ($P < 0.05$) for cows fed the maize diet (MF and MC) than cows fed sorghum diets (SF and SC), potentially indicating an improvement in ruminal nitrogen utilization in the maize diets. Although sorghum diets had a higher concentration of CP, the intake was lower as compared to maize diets. No difference on MUN was observed between particle size i.e. fine maize and coarse maize nor between fine sorghum and coarse sorghum. Fredin et al. (2015) and Brossillon et al. (2018) found that MUN concentration was not affected by differences in maize particle size. However, Eastridge et al. (2011) found that finely ground maize decreased MUN concentration compared with coarsely ground maize, suggesting that higher ruminal starch digestibility possibly improved bacterial capture of the nitrogen. Concurring with Eastridge et al. (2011), Ferraretto et al. (2013) reported that MUN concentration tended to increase with increasing mean particle size. Feed efficiency (ECM/DMI) was affected by dietary treatment, with greater efficiency for cows fed sorghum diets than maize diets, which could be because of the lower DMI.

Overall, greater differences in particle sizes are likely to affect production and milk composition as observed by Knowlton et al. (1996) with the average differences of 2,438 μm between ground and cracked maize compared to the average differences of 718 μm reported by Fredin et al. (2015). Knowlton et al. (1996) reported no effects on DMI and yield of milk fat but milk yield tended to increase, and milk protein increased in ground maize as compared to cracked maize, whereas Fredin et al. (2015) observed no effects of particle size on DMI, milk yield, and concentration and yield of milk components. Similar

results were reported by Brossillon et al. (2018), although the magnitude of the difference was higher (averaged 1,467 μm) between ground and cracked maize. The NGMPS observed for finely and coarsely ground grains in the present study were 255.55 and 550.14, 250.62 and 728.54 μm for maize and sorghum, respectively (Table 6.2), with differences averaging 294.59 and 477.92 μm between finely and coarsely ground maize and sorghum, respectively. Nevertheless, in a meta-analysis with mean particle size differences of 500 to 3,500 μm , Ferraretto et al. (2013) reported no differences in DMI, milk yield and milk components, and feed efficiency between particle sizes.

Table 6. 4. Dry matter intake (DMI), milk yield and milk composition of lactating dairy cows fed maize or sorghum varying in particle sizes.

Item	Maize		Sorghum		SEM	P-value
	Fine	Coarse	Fine	Coarse		
DMI, kg/d	25.50	24.81	22.58	21.39	1.50	0.1558
MY, kg/d	25.27	23.81	25.37	22.51	1.59	0.0603
BW, kg	723.02	706.14	720.67	692.79	24.52	0.2529
DMI, % of BW	3.55	3.64	3.14	3.12	0.24	0.2276
ECM, ¹ kg/d	27.86	27.25	28.86	26.22	1.80	0.5166
Feed efficiency ²	1.11 ^b	1.10 ^b	1.28 ^a	1.23 ^a	0.06	0.0240
Milk fat, %	4.02	4.34	4.33	4.52	0.24	0.5583
Milk fat, kg/d	1.01	1.03	1.09	1.01	0.08	0.8246
Milk protein, %	3.58	3.60	3.56	3.58	0.07	0.9593
Milk protein, kg/d	0.90	0.85	0.90	0.80	0.05	0.2335
Milk lactose, %	4.80	4.72	4.65	4.60	0.07	0.1378
Milk lactose, kg/d	1.22	1.13	1.19	1.04	0.09	0.1145
MUN, mg/dL	14.36 ^b	14.89 ^b	16.99 ^a	17.09 ^a	0.81	0.0030

^{a-b}values within a row with different superscript differ ($P < 0.05$).

¹ECM (energy-corrected milk) = $[0.327 \times \text{milk yield (kg)}] + [12.95 \times \text{fat yield (kg)}] + [7.2 \times \text{protein yield (kg)}]$ (Orth, 1992)

²Feed efficiency = ECM/DMI

DMI = Dry matter intake; MY = Milk yield; MUN = Milk urea nitrogen; BW = Body weight

6.4.4. Characteristics of ruminal fermentation

Dietary treatment effects on mean ruminal and reticular pH, ammonia nitrogen concentrations ($\text{NH}_3\text{-N}$), total VFA concentration, and VFA molar proportions are presented in Table 6.5. Rumen and reticulum pH were greater ($P < 0.05$) on the SC diets compared to other treatments, likely as a result of lower rumen starch digestibility. Fredin et al. (2015) and Eastridge et al. (2011) reported a greater rumen pH for the coarsely

ground maize diets than for finely ground maize, but other studies have reported no influence of grain particle size on rumen pH (Knowlton et al., 1996, 1998; San Emeterio et al., 2000; Callison et al., 2001; Rémond et al., 2004). Decreased ruminal pH in finely ground grains could possibly be explained by increased ruminal starch digestibility with decreasing particle size, increasing VFA production, potentially resulting in lower pH (Fredin et al., 2015). The minimum (maximum) rumen pH of the present study was 5.53 (6.52), 5.49 (6.31), 5.47 (6.63) and 5.89 (6.61) for MF, MC, SF and SC, respectively. The lowest pH was recorded at 0800h after the morning feeding (0730h). The values for reticular pH were 5.79 (6.79), 6.03 (6.66), 5.93 (6.89) and 6.61 (6.94) for MF, MC, SF and SC, respectively. Although the rumen and reticular pH fluctuated across sampling time (Figure 6.1 and 6.2, respectively) with some ruminal pH below 5.8, the average ruminal pH for all treatments were above 5.8, which is considered as a threshold for rumen acidosis (Penner et al., 2007). The risk of subacute rumen acidosis is known to increase when ruminal pH drops below 5.8 for more than 5-6 h/day (Zebeli et al., 2012). The reticular pH was consistently higher than the ruminal pH over sampling time, with the mean pH of 6.02 and 6.40 for the rumen and reticulum, respectively.

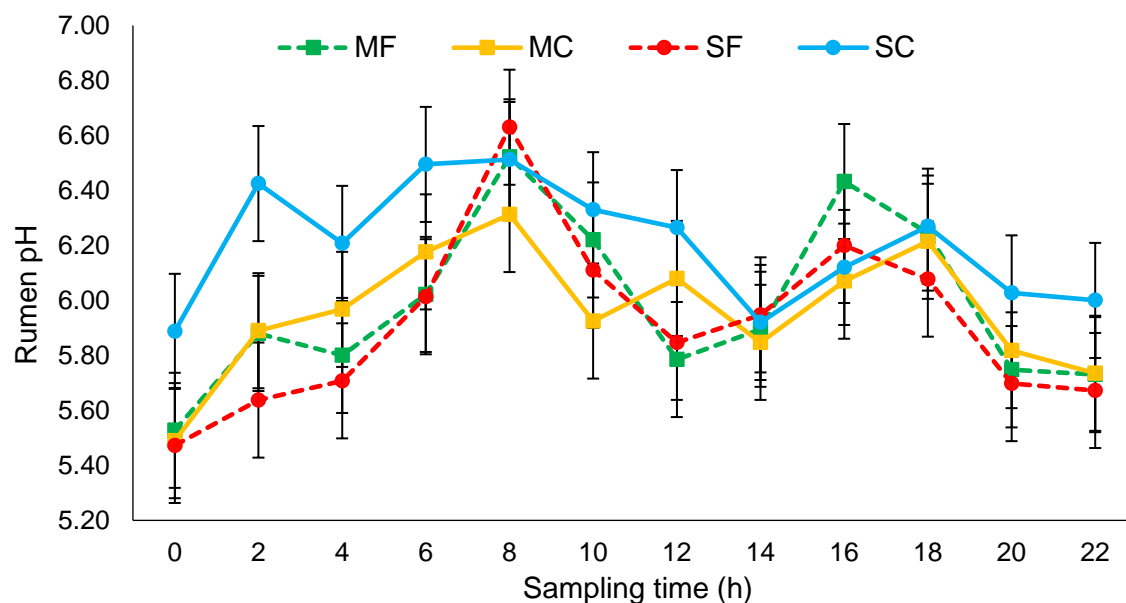


Figure 6. 1. Effects of particle size of maize and sorghum diets on rumen pH over sampling time.

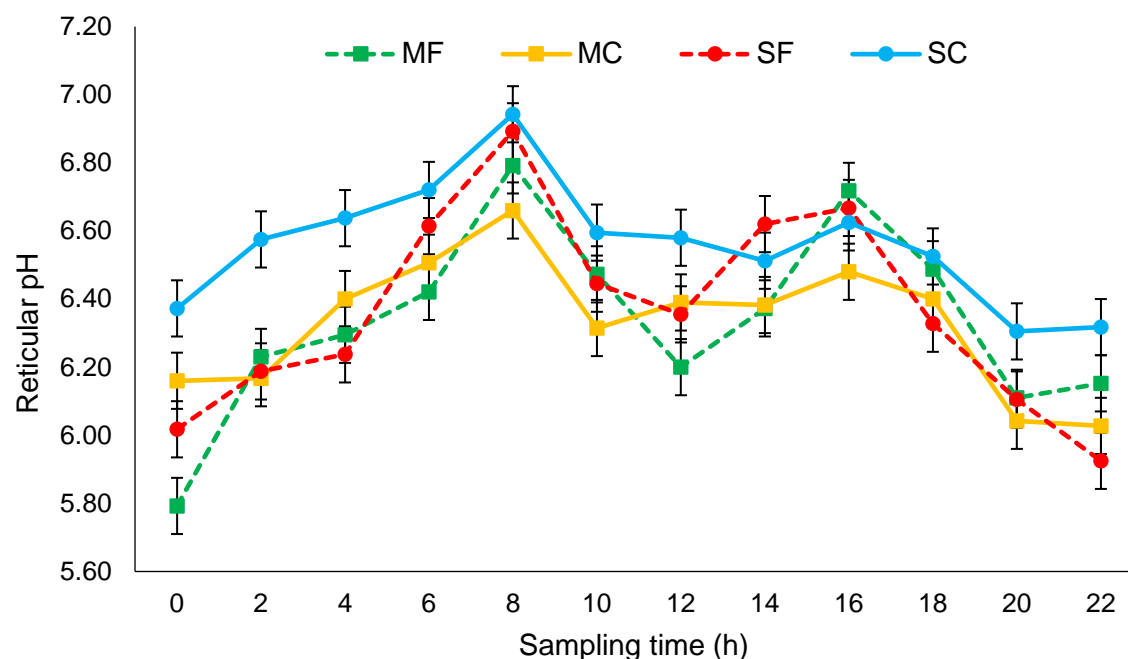


Figure 6. 2. Effects of particle size of maize and sorghum diets on reticular pH over sampling time.

Rumen $\text{NH}_3\text{-N}$ (mg/dL) was lower for cows fed MF compared with cows fed SF and SC. Cows fed fine maize tended to lower rumen $\text{NH}_3\text{-N}$ than for cows fed coarse maize ($P = 0.08$). The ruminal concentration of total VFA (mM) was affected by dietary treatment ($P = 0.0158$), with lower VFA on the SC diet compared with other treatments. Eastridge et al. (2011) and Rémond et al. (2004) reported lower ruminal $\text{NH}_3\text{-N}$ concentration for finer particle sizes than coarser particles. Other researchers (Knowlton et al., 1998; Fredin et al., 2015) reported that the rumen $\text{NH}_3\text{-N}$ (mg/dL) and total VFA (mM) were unaffected by differences in particle size. According to the literature, lower ruminal $\text{NH}_3\text{-N}$ concentration for finer vs. coarser particles might be associated with greater ruminal starch degradation possibly resulting in increased utilization of nitrogen by the microbes (Placenia and Zinn, 1995). The lower VFA concentration and greater rumen pH in cows fed SC suggests lower ruminal starch digestibility than other diets.

The concentration of ruminal propionate was lower for cows fed the SC diet compared to other treatments ($P < 0.0001$). Both maize diets had greater propionate concentration than sorghum diets, attributed to starch digestibility. Starch in sorghum has a lower digestibility compared to other cereals because of a strong starch-protein matrix

(Herrera-Saldana et al., 1990; Kotarski et al., 1992). Ruminal acetate was lower for the MF diet compared with the MC diet ($P = 0.009$), resulting in an increase in the acetate:propionate ratio for the MF diet compared to the MC diet ($P < 0.0001$). On the other hand, there was no difference in ruminal acetate between cows fed the SF and SC diet (65.83 vs. 64.08 mol/100 mol), but the acetate: propionate ratio was decreased for cows fed the SF diet, possibly because of increased ruminal propionate for cows fed the SF diet compared to the SC diet (31.22 vs. 28.68 mol/100 mol). The lower acetate:propionate ratio observed in the MF diet compared with other diets can be attributed to higher ruminal starch digestibility, resulting in higher propionate concentration as compared to other diets. The increased ruminal starch availability usually results in higher propionate concentrations and lower molar ratios of acetate to propionate (Placencia and Zinn, 1995). Butyrate was greater for the SF as compared with the SC and MF diets, whereas isobutyrate was lower in the MF diet ($P = 0.0022$) compared with both sorghum diets. Valerate and isovalerate were greater in cows fed SF compared to the SC, but no differences were observed between MF and MC diets. The increased concentration of rumen propionate and decreased concentration of isobutyrate and acetate:propionate ratio observed on the MF diet concurs with other studies. Although some studies have reported that rumen propionate is unaffected by particle size (Knowlton et al., 1996, 1998; Rémond et al., 2004), others have reported that finely ground maize increased ruminal propionate, decreased isobutyrate and the ratio of acetate to propionate when compared to coarsely ground maize (Knowlton et al., 1996; Eastridge et al., 2011; Fredin et al., 2015), reflecting a greater extent of ruminal degradation with finely ground grains compared to coarsely ground grains. On the contrary, increased rumen pH, decreased ruminal propionate, and increased acetate:propionate ratio for the cows fed SC diet would suggest a lower ruminal starch digestibility for the SC diets compared with the other diets.

Table 6. 5. Ruminal and reticular pH, ruminal NH₃-N concentration and VFA concentrations of lactating dairy cows fed maize or sorghum varying in particle size.

Item	Maize		Sorghum		SEM	P-value
	Fine	Coarse	Fine	Coarse		
Rumen pH,	5.98 ^b	5.96 ^b	5.92 ^b	6.20 ^a	0.16	0.0014
Reticular pH	6.33 ^b	6.32 ^b	6.36 ^b	6.56 ^a	0.14	0.0009
NH ₃ -N, mg/dL	15.97 ^b	18.06 ^{ab}	18.56 ^a	18.82 ^a	2.11	0.0311
Total VFA, mM	112.05 ^{ab}	115.90 ^a	113.57 ^a	108.34 ^b	4.45	0.0158
VFA molar proportions, mol/100 mol						
Acetate	62.80 ^b	67.02 ^a	65.83 ^{ab}	64.08 ^{ab}	1.42	0.0453
Propionate	33.87 ^a	32.96 ^a	31.22 ^b	28.68 ^c	2.78	<0.0001
Acetate: Propionate	1.91 ^d	2.10 ^c	2.21 ^b	2.33 ^a	0.18	<0.0001
Butyrate	8.88 ^{bc}	9.15 ^{ab}	9.47 ^a	8.74 ^c	0.26	0.0002
Isobutyrate	2.39 ^b	2.51 ^{ab}	2.63 ^a	2.65 ^a	0.08	0.0022
Valerate	2.55 ^{ab}	2.58 ^{ab}	2.62 ^a	2.47 ^b	0.20	0.0305
Isovalerate	1.57 ^c	1.63 ^c	1.80 ^a	1.71 ^b	0.04	<0.0001

^{a-b} values within a row with different superscript differ ($P < 0.05$).

6.4.5. Digestibility and nutrient flow

Treatment effects on intake, flow and digestibility of DM, OM, starch and NDF are presented in Table 6.5. Dry matter and NDF intake were not affected by dietary treatments, while OM and starch intakes differed among diets. Cows fed the SC diet had a lower intake of OM compared with cows fed the MF diet because of the differences in DMI (21.30 vs. 25.50 kg/d). Intake of starch was greater for cows fed the MF and MC diets (6.77 and 6.50 kg/d, respectively) compared with cows fed the SF and SC diets (5.68 and 5.26 kg/d, respectively) ($P=0.0119$). The differences in starch intake could be attributed to DMI because starch and DM intake are positively correlated (Moharrery et al., 2014). In the present study, cows fed maize diets had a numerically greater intake than cows fed sorghum diets (average 25.16 vs. 21.99 kg/d). Specifically, DM, OM, NDF and starch intake did not differ between cows fed finely and coarsely ground grains of the same cereal (i.e. maize or sorghum), but cows fed the sorghum diet had a lower starch intake which can be explained by its lower starch content in addition to the lower DMI. In agreement with our results, Fredin et al. (2015) found that DM, OM, NDF and starch intakes were unaffected by particle size. Callison et al. (2001) and Rémond et al. (2004)

found that the nutrient intake was similar for finer ground maize and coarsely ground maize.

Apparent ruminal digestion of OM and DM was greater for cows fed the MC diet compared with cows fed the SF diet (52.96 vs. 44.47% and 44.28 vs. 33.89%, respectively), thereby decreasing the amount of OM and DM flow at the omasum. The flow of DM at the reticulum was greater for cows fed the SF diet compared with the SC diet (14.86 vs. 12.67 kg/d) but comparable against the MF and MC diets (14.73 and 13.80 kg/d, respectively). OM and NDF flow, however, did not differ among diets ($P > 0.10$). The apparent ruminal digestion of NDF differed among diets ($P = 0.0396$), with lower digestibility for cows fed the MF diets compared to cows fed the SC diets (28.32 vs. 36.57%). This can be explained by the higher rumen starch digestibility of MF compared to SC (88.77 vs. 83.10%). Increased dietary starch combined with more rapid rumen starch digestibility decreases rumen pH (Nocek and Tamminga, 1991). This will lead to an unfavourable environment for cellulolytic bacteria growth and adherence thereby decreasing NDF digestibility (Firkins et al., 2001; Ferraretto et al., 2013). In this study, there was no significant difference between ruminal digestibility of NDF for finely and coarsely ground maize nor between the finely and coarsely ground sorghum treatments. Similarly, Fredin et al. (2015) found that apparent ruminal digestibility and flow at the reticulum of OM and NDF were unaffected by maize grain particle size.

Apparent ruminal starch digestion of was affected by dietary treatment, with greater ruminal starch digestion observed for cows fed the MF diet compared with the MC diet (88.77 vs. 83.76%). Although the apparent ruminal digestion did not differ among the SF and SC diets, the amount of starch digested ruminally was numerically decreased for SC compared with that of the SF diet (83.10 vs. 85.59 %). A greater digestibility of starch in the rumen for finely ground grains could be attributed to increased surface area for microbial attack (Huntington, 1997). Starch digestibility increases as particle size decreases (Ferraretto et al., 2013). As a result, it was observed that the starch flow at the reticulum was greater for cows fed the MC than MF diet, indicating that coarser particles decreased the amount of starch digested ruminally, and thereby increasing the amount of starch escaping to the small intestine. In agreement with our results, using duodenally cannulated cows Rémond et al. (2004) found that ruminal starch digestibility decreased

for coarsely rolled maize as compared to ground maize (53.5 vs. 69.8%). Surprisingly, using the reticular sampling technique, Fredin et al. (2015) found greater apparent ruminal digestion of starch for coarser maize particles than finer particles, resulting in a reduced amount of starch flowing to the small intestine. Similar to Fredin et al. (2015), in another study using duodenally cannulated cows, Knowlton et al. (1998) reported increased ruminal starch digestion for coarser than finer maize particle size (69.2 vs. 60.9%).

Apparent ruminal starch digestion averaged at 88.77, 83.76, 85.59 and 83.10 % for MF, MC, SF and SC, respectively. These results were within a normal expected value range (72.0 to 89.9%) (Huntington, 1997). Fredin et al. (2015) reported apparent rumen starch digestibility ranging from 71.2 to 83.7% in cows fed finely and coarsely ground maize. Lower values (53.5%) have however also been reported in a meta-analysis by Ferraretto et al. (2013). Depending on several factors as described by Giuberti et al. (2014), ruminal starch digestibility can be highly variable, ranging from 22.4 to 94.2% (Moharrery et al., 2014).

The apparent total-tract digestibilities of DM and OM were unaffected by dietary treatment ($P > 0.10$). In agreement, Fredin et al. (2015) reported that total-tract digestion of DM and OM were unaffected by particle size. Similarly, Yu et al. (1998) found that total-tract OM digestibility was unaffected by particle size. However, Brossillon et al. (2018) reported that apparent total-tract digestibilities of DM and OM were greater for cows fed ground maize than cracked maize. Concurring, Knowlton et al. (1998) reported greater total-tract DM digestibility (62.0 vs. 58.9%) and Rémond et al. (2004) reported greater total-tract OM digestibility (73.3 vs. 68.3%) for cows fed finely ground maize than coarsely ground maize. Moreover, in a meta-analysis, Ferraretto et al. (2013) reported that the apparent total-tract digestibilities of DM and OM increased as maize grain particle size decreased. Total-tract NDF digestibility (TTNDFD) was lower for MF compared with SC (35.15 vs. 41.84%). This is likely related to total tract starch digestibility as ruminal starch digestion negatively affects ruminal fibre digestion (Oba and Allen, 2003). There were no differences observed between finely ground grains of the same cereal. Previous studies found that the TTNDFD was unaffected by particle size (Fredin et al., 2015; Brossillon et al., 2018). Similarly, the meta-analyses by Firkins et al. (2001) and Ferraretto et al. (2013) reported similar TTNDFD for coarse and finely ground maize. However, Yu et al. (1998)

found lower NDF digestibility for finely ground maize than coarse particle (62.8 to 54.4%), which was negatively related to starch digestibilities. The average TTNDF digestibility across diets in the present study was 38.5%. This is lower than averages (46%) reported by Brossillon et al. (2018) and Fredin et al. (2015), possibly due to starch digestibility. A meta-analysis by Ferraretto and Shaver (2012) using a data set composed of 106 treatments from 24 peer-reviewed articles reported that the TTNDFD averaged 44 ± 2.5 %.

Table 6. 6. Intake, flow and digestibility of nutrients in the lactating dairy cows fed maize or sorghum varying in particle sizes.

	Maize		Sorghum		SEM	P-value
	Fine	Coarse	Fine	Coarse		
Item*						
DM						
Intake, kg/d	25.50	24.81	22.58	21.39	1.50	0.1558
Apparent ruminal digestion, %	41.44 ^{ab}	44.28 ^a	33.89 ^b	39.94 ^{ab}	2.91	0.0464
Flow at reticulum, kg/d	14.73 ^{ab}	13.80 ^{ab}	14.86 ^a	12.67 ^b	0.66	0.0397
Apparent total-tract digestion, %	63.26	59.10	61.77	58.49	2.62	0.5681
OM						
Intake, kg/d	23.70 ^a	23.06 ^{ab}	20.97 ^{ab}	19.83 ^b	1.40	0.0259
Apparent ruminal digestion, %	49.81 ^{ab}	52.97 ^a	44.47 ^b	50.45 ^{ab}	2.83	0.0442
Flow at reticulum, kg/d	11.70	10.82	11.64	9.71	0.63	0.1621
Apparent total-tract digestion, %	64.91	60.37	63.65	59.54	2.67	0.4817
Starch						
Intake, kg/d	6.77 ^a	6.50 ^a	5.68 ^b	5.26 ^b	0.41	0.0119
Apparent ruminal digestion, %	88.77 ^a	83.76 ^b	85.59 ^{ab}	83.10 ^b	1.36	0.0409
Flow at reticulum, kg/d	0.76 ^b	1.04 ^a	0.82 ^b	0.88 ^{ab}	0.05	0.0255
Apparent total-tract digestion, %	96.29 ^a	87.84 ^b	92.21 ^{ab}	86.93 ^b	2.36	0.0280
NDF						
Intake, kg/d	7.84	7.86	6.76	6.58	0.56	0.3105
Apparent ruminal digestion, %	28.32 ^b	33.18 ^{ab}	32.14 ^{ab}	36.57 ^a	1.65	0.0396
Flow at reticulum, kg/d	5.59	5.26	4.64	4.18	0.46	0.2056
Apparent total-tract digestion, %	35.15 ^b	37.65 ^{ab}	39.29 ^{ab}	41.84 ^a	1.53	0.0458

^{a-b}values within a row with different superscript differ ($P < 0.0001$).

*DM= dry matter; OM = organic matter; NDF= Neutral detergent fiber; CP= crude protein.

Total-tract digestion of starch was higher for cows fed the MF diet compared with cows fed the MC diet (96.29 vs. 87.84%). Despite no significant differences observed between the SF and SC diets, total tract starch digestibility (TTSD) was numerically greater for cows fed SF as compared with SC (92.21 vs. 86.93%). The finer particles

increased starch digestibility by 8.5 and 5.3 percentage units in the maize and sorghum diets, respectively. Most previous studies reported increased TTSD for cows fed finely ground diets (Knowlton et al., 1996, 1998; Yu et al., 1998; Rémond et al., 2004; Fredin et al., 2015; Brossillon et al., 2018). Brossillon et al. (2018) and Yu et al. (1998) reported that TTSD increased by 8 percentage units in cows fed ground maize as opposed to cracked maize (97.6 vs. 89.2 % and 95.8 vs. 87.4%, respectively), whereas Knowlton et al. (1998) reported an increase of 12.5 percentage units (88.9 vs. 76.4%). Fredin et al. (2015) found that TTSD was affected by particle size, with the largest differences observed in normal starch-finely ground maize, which was increased by 7 percentage units (i.e. 98.1 vs. 91.1%, finely and coarsely ground maize). In a meta-analysis, Ferraretto et al. (2013) and Firkins et al. (2001) reported that TTSD increased from 78 to 93 % and 85 to 92%, respectively as maize grain particle size decreased. The greater TTSD of finer particles is attributed to its increased surface area, thereby facilitating ruminal microbes and enzymatic digestion compared with coarser particles (Huntington, 1997).

The mean TTSD values were 96.29, 87.84, 92.21 and 86.93% for MF, MC, SF and SC, respectively, which are comparable with the expected values reported in the literature. Starch digestibility is however, highly variable as indicated by a meta-analysis, Moharrery et al. (2014) who summarized that TTSD averaged 93.1%, ranging from 69.4 to 100%, depending on starch sources.

6.5 Conclusion

In the present experiment DMI, milk yield and composition were not affected by dietary treatments, however, MUN was lower for cows fed maize compared to sorghum diets. Rumen and reticulum pH were greater for the sorghum coarse diet compared to other diets. Propionate concentration was greater for both maize diets, however finely ground maize had lower acetate concentration, thereby decreasing the acetate to propionate ratio. Ruminal ammonia nitrogen tended to be lower for the fine maize diet compared to other diets. Dietary treatments did not affect OM and NDF intake, flow of DM, OM and NDF, or ruminal digestibility of OM. Starch intake, flow and ruminal digestibility differed among diets. Ruminal starch digestibility was lower for cows fed

coarsely ground grains, resulting in increased starch flowing to the abomasum, however total tract starch digestibility was decreased. Furthermore, cows fed the finely ground maize had a higher ruminal starch digestibility, but a lower ruminal NDF digestibility compared to cows fed the coarsely ground sorghum diet. This study confirms that coarser particles can allow some of the starch digestion to be shifted from the rumen to the small intestine. It may, however, reduce total starch digestibility if ruminal digestion is not compensated for by small or large intestinal digestion. The results of this study did not provide evidence to support the HOT, given that the higher propionate production in cows fed finer maize did not decrease feed intake in the late lactation. Further research is needed to investigate the effects of altering starch fermentability by particle size on DMI and production of transition cows to develop dietary starch strategies to minimize negative effects associated with the transition period.

6.7 References

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CHAPTER 7

Effects of starch fermentability of fresh cow diets on feeding behaviour, feed intake, lactation performance and metabolic status of dairy cows in the early postpartum period

7.1. Abstract

The objective of this study was to evaluate the effects of starch fermentability of diets fed during the early postpartum (PP) period on feeding behaviour, dry matter intake (DMI), lactation performance and body metabolism of fresh dairy cows. A total of 117 Jersey cows were used in a randomized block design. Treatment diets were fed as a total mixed ration (TMR) from calving to 30 d PP and formulated to contain 28 % of starch, with varying particle size of ground maize (3mm or 6 mm screen sieve), as the primary source of starch. Throughout the experiment DMI, milk yield and body weight were measured daily, milk composition, body condition score (BCS) and blood metabolites were recorded weekly. Feeding coarsely ground maize (MC) increased dry matter intake (16.08 vs. 17.13 kg/d) and milk yield (20.41 vs. 21.70 kg/d) during the early PP period compared to finely ground maize (MF). Diet did not affect eating (581.13 vs. 583.57 min/d) and rumination time (308.58 vs. 315.35 min/d, MF vs. MC) across the whole period. The lower DMI in cows fed finely ground maize compared to cows fed coarsely ground maize supports the hepatic oxidation theory of the control of feed intake. Diets had no effect on yields of milk protein, ECM, 3.5 % FCM or MUN (0.74 and 0.76 kg/d, 26.91 and 27.67 kg/d, 27.07 and 27.75 kg/d, and 9.61 and 10.28 mg/dL, respectively for the MF and MC diets). Milk lactose was increased with MC compared to the MF diet (4.61 vs. 4.70 %). Milk fat percentage tended to be greater (5.57 vs. 5.27%) with MF compared to the MC diet, but milk fat yield did not differ (1.12 vs. 1.13). Changes in BW and BCS were greater in cows fed finely ground maize (39.92 vs 32.24 kg and 0.23 vs. 0.14 units). Feeding the MC diet decreased the concentration of plasma NEFA (0.71 vs. 0.56 mmol/L), suggesting that these cows were in a better metabolic status as they could reduce the mobilization of body reserves. Blood glucose levels were not affected by treatment.

7.2. Introduction

During the transition period (3 weeks prior to 3 weeks after calving), dairy cows experience a major increase in demand for energy (by two to three-folds; Drackley et al., 2001; Reynolds et al., 2003). This combined with a dramatic reduction in dry matter intake (~30%) (Bertics et al., 1992; Grummer, 1993) lead to a negative energy balance (NEB), which is associated with an increase in metabolic and health problems, reduction in milk production as well as in reproductive performance (Grummer, 1995; Goff and Horst, 1997; Drackley, 1999; Butler, 2003; Mulligan and Doherty, 2008; LeBlanc, 2010; Esposito et al., 2014). The early postpartum (PP) (2-3 weeks postpartum) period is considered as a major risk period. This period is normally categorized by high incidence of metabolic disorders, severe energy deficiency and excessive lipid mobilization (Overton and Waldron, 2004; Esposito et al., 2014). This is because a reduction in DMI is intensified during the early PP period and is often inadequate to support the rapid increase in the energy demand for milk production (Bell, 1995; Drackley, 1999). In an effort to minimize these effects as well as to support milk production, fresh cows are typically fed diets high in starch ($\pm 30\%$ DM basis).

Starch sources fed to dairy cows vary greatly with regards to ruminal fermentability (<30 to >90%; Moharrery et al., 2014). Furthermore, according to the hepatic oxidation theory (HOT), highly fermentable starch sources increase the production of propionate which can suppress feed intake (Allen, 2000; Oba and Allen, 2003a; Bradford and Allen, 2007; Allen et al., 2009). Although propionate from ruminal fermentation of starch is a primary glucose precursor, it is hypophagic (Forbes, 2007; Gualdrón-Duarte and Allen, 2018; Maldini and Allen, 2018). Variation in the ruminal starch digestibility of diets fed to dairy cows results in different rates of propionate production, absorption and flux to the liver (Gualdrón-Duarte and Allen, 2018). Moreover, during the early PP period cows in a lipolytic state mobilize body reserves, elevating the concentration of plasma NEFA, which intensifies the hypophagic effects of propionate (Oba and Allen, 2003b; Allen et al., 2009; Stocks and Allen, 2012, 2013; Piantoni et al., 2015).

The HOT suggests that the site of starch digestion can modulate the negative effects associated with increased rumen starch fermentation such as feed intake, and the occurrences of problems encountered by transition cows. However, the effects may vary

across lactation (Allen et al., 2009). For instance, when the site of starch digestion is shifted postruminally, fuel absorbed postruminally would not stimulate hepatic oxidation to the same extent as propionate. Moreover, because of increased latency for fuels reaching the liver; transit time from the rumen to the small intestine significantly delays fuel absorption (Albornoz and Allen, 2018). Larsen et al. (2009) suggested that feeding rations that partly shift the digestion of starch from the rumen to the small intestine is an attractive strategy to overcome some of the nutritional shortcomings associated with meeting the nutrient needs of transition cows. Therefore, the effects of the concentration and fermentability of starch in rations of lactating cows especially during the early PP period need to be well understood for better formulation of fresh cow rations.

The effects of dietary starch concentration during the early PP period has been extensively studied and varying effects on DMI and performance of dairy cows was observed (Andersen et al., 2003; Rabelo et al., 2003, 2005; Dann and Nelson, 2011; McCarthy et al., 2015a; b; Williams et al., 2015; Albornoz and Allen, 2018). Limited studies on the effects of ruminal fermentability of starch in diets fed to cows during the early PP period (Dann et al., 1999; Sadri et al., 2009; Albornoz and Allen, 2018) are published. Results have further been inconsistent (Dann et al., 1999; Sadri et al., 2009; Albornoz and Allen, 2018). Concurring with the hepatic oxidation theory of the control of feed intake (Allen et al., 2009), some studies reported that increasing ruminal starch fermentability decreased DMI (Dann et al., 1999; Sadri et al., 2009; Albornoz and Allen, 2018). Rockwell and Allen (2016), in contrast, reported no effect on DMI. In these previous studies, ruminal starch fermentability was increased by substituting steam-flaked maize for cracked maize (Dann et al., 1999), ground barley for ground maize (Sadri et al., 2009) or high-moisture maize for dry ground maize (Rockwell and Allen, 2016; Albornoz and Allen, 2018). The effects of increasing starch fermentability by varying the particle size of the starch source in diets fed to cows in the early PP period is not known. Typically, cereal grains are milled prior to inclusion in diets of dairy cows to reduce their particle size, increasing the surface area, hence the degradability. Particle size of grains affects rumen starch fermentability (Ferraretto et al., 2013), and can modulate the site of starch digestion (Shipandeni et al., 2018).

The objective of this study was to evaluate the effects of starch fermentability by varying the maize particle size of the diet fed during the early postpartum (PP) period on feeding behaviour, dry matter intake (DMI), lactation performance and metabolism. We hypothesized that diets with coarsely ground maize would reduce fermentation of starch in the rumen, hence the production of propionate, thereby limiting the reduction of DMI and possibly improving lactation performance of fresh cows compared with diets with finely ground maize, which are highly fermentable.

7.3. Materials and Methods

Animal care and all experimental procedures carried out in this experiment were approved by the Stellenbosch University's Research Ethics Committee: Animal Care and Use (REC: ACU) (protocol number: 0944). The experiment was conducted from May to July 2018 at Wydgelegen dairy farm, Western Cape, South Africa. The mean minimum and maximum temperatures were 8.3 °C and 21.9 °C, respectively, and the total rainfall during the trial period was 56.4 mm (Agricultural Research Council, 2018).

7.3.1 Cows, experimental design and dietary treatments

A total of 117 Jersey cows (52 primiparous and 65 multiparous) were selected from the dairy herd based on their 305 d-milk yield and lactation number (≤ 5) for multiparous cows. The selected cows were blocked by parity (primiparous or multiparous) and balanced for expected calving date, milk yield, body weight and body condition score and randomly assigned to 1 of the 2 experimental diets. Lactating cows were dried off at least 60 days before expected calving date. A common close-up diet based on oat silage and wheat straw was fed from 21 d before expected calving date until calving. The close-up diet was formulated to contain 13.3% starch, 47.9% NDF and 15.8 % CP.

The experimental diets were a finely ground maize as a control (3 mm ground maize; MF) or coarsely ground maize as a treatment (6 mm ground maize; MC) diet. The control diet reflects a typical diet fed to lactating dairy cows on many commercial farms in South Africa. The treatment diet was selected based on the results from our preliminary study (Shipandeni et al., 2018) to intentionally shift the site of starch digestion, by altering rumen fermentability. Maize (*Zea mays*) grain was sourced locally and milled at

Wydgelegen dairy farm using a Drotsky hammer mill fitted with a 3 or a 6-mm screen sieve, representing fine and coarse particle sizes, respectively. Samples of both milled maize were taken for chemical composition and 7-h *in vitro* starch degradability analysis according to Goering and Van Soest (1970). Rates of starch degradation were then computed using the 7 h formula (Sniffen and Ward, 2011) as follows:

$$K_d-7 = (((LN(\text{Starch, \%DM})) - (LN(((100 - 7h \text{ ivSd, \%Starch}) / 100) * \text{Starch, \%DM}))) / 7) * 100,$$

where starch% is the starch content of the grain on DM and ivSd% is the *in vitro* starch degradability as % of starch. The *in vitro* starch degradability and rate of starch degradation are presented in Table 7.1.

Table 7. 1. Nutrient composition, degradability and rate of starch degradation of maize milled using a 3- and 6-mm screen.

	Maize	
	3 mm	6 mm
Nutrient composition ¹ , % DM		
DM	87.87	87.72
OM	99.39	99.39
Starch	73.05	70.21
CP	9.42	9.11
Ash	0.61	0.61
EE	4.21	3.82
NDF	14.36	22.72
ADF	4.21	5.72
Starch digestibility, 7 h, ² %	65.32	58.02
k _d , ³ % h ⁻¹	15.14	12.46

¹DM=Dry matter; OM=Organic matter; CP=Crude protein; EE=Ether extract; NDF=Neutral detergent fibre; iNDF=indigestible NDF; ADF=Acid detergent fiber.

²Starch degradability measured *in vitro* after 7-h incubation.

³k_d = rate of starch degradation.

Cows were housed in groups in four pens as control cows, treatment cows, control heifer and treatment heifer, whereby the control groups were assigned to a 3 mm ground maize and the treatment groups to a 6 mm ground maize. The pens were outdoor, bedded with sand and equipped with feed and water troughs. Cows were kept in a colostrum pen

for the first 5 days after calving prior to their respective pens where they received their assigned diet. Cows were allowed to move freely within their respective pens. Daily observations and general health records were maintained throughout the study.

Experimental diets, formulated to meet or exceed nutrient requirements recommended by the NRC (NRC, 2001) and the respective ingredient and nutritional composition are presented in Table 7.2. Oat silage (DM) contained 8.3% CP, 54.5% NDF, 39.9% ADF, 7.4% ADL, 6.8% starch and 3.6% EE. For lucerne hay the respective values were 16.5%, 43.3%, 30.0%, 7.3%, 1.27 and 1.6%. All feed ingredients, maize, oat silage, wheat straw, lucerne and a high protein concentrate (Afgri HPC; Afgri Animal Feeds, Centurion, GP, South Africa), with addition of water, were mixed in a feed mixer wagon (Rumax 15) directly prior to feeding. Diets were formulated to contain 28 % starch, which is typically fed during the postpartum period on many commercial dairy farms in South Africa.

The experimental diets were fed as a total mixed ration (TMR) twice daily for *ad libitum* intake at 0600 and 1600 h from day 5 after calving to 30 days in milk (DIM). The amounts of TMR offered were adjusted daily ensuring that cows do not empty feed troughs. Feed adjustment was based on the intake of the previous day, allowing at least 5% refusals per pen. The DMI (kg/d) was determined on a per pen basis by recording the amount of feed offered and the amount of feed refused each morning before new feed delivery throughout the entire experiment. The total number of cows in a pen was also recorded daily. Water was available *ad libitum*. After 30 DIM, cows were moved back to their customary groups and fed their common diets following standard farm procedure.

Table 7. 2. Ingredients and nutrient composition of experimental diets fed as TMR.

Items	Fresh cow diet	
	Fine ground diet	Coarsely ground diet
Ingredients, % of DM		
Oat silage	40.68	40.68
Lucerne	10.17	10.17
Wheat straw	3.39	3.39
Commercial HPC ¹	19.32	19.32
Ground maize (3 mm)	26.44	-
Ground maize (6 mm)	-	26.44
Nutrient composition*, % of DM		
DM	47.17	47.25
OM	91.81	92.74
Starch	25.44	27.72
CP	17.19	17.43
EE	2.70	2.59
NDF	35.19	35.91
iNDF	12.85	12.40
ADL	3.12	3.21
pdNDF ³	22.34	23.51
NFC ⁴	36.74	36.81
Ash	8.19	7.26

¹HPC (high protein concentrate) was sourced from Afgri (Afgri Animal Feeds, Centurion, GP, South Africa), contained wheat bran, gluten 21, chop, soya oil cake, molasses, poultry blood meal, canola oil cake, sunflower oil cake, savanna chalk, urea, salt.

*DM=Dry matter; OM=Organic matter; CP=Crude protein; EE=Ether extract; NDF=Neutral detergent fibre; ADL=Acid detergent lignin; iNDF=indigestible NDF; pdNDF=potentially digestible NDF; NFC=Non-fibrous carbohydrates.

³ Calculated as NDF – iNDF.

⁴ Calculated as 100 – (CP % + NDF % + ether extract % + ash %); NRC, 2001.

7.3.2 Sampling procedures and data collection

7.3.2.1 TMR offered and orts samples: Samples of TMR offered and orts were obtained weekly throughout the trial and stored at –20°C for subsequent analysis. After the trial, TMR offered and orts samples were thawed at room temperature, composited per camp and a subsample was dried in a forced-air oven at 60 °C for 72 h to determine DM content of the diets. Prior to analysis (DM, ash, starch, ether extract, CP, NDF, ADL and indigestible NDF (iNDF)), dried samples were ground to pass through a 1-mm sieve using a Wiley mill (Model 4, Thomas-Scientific, USA). To determine the DMI, feed offered and refusals were recorded daily per pen; group feed intake was calculated by difference assuming a similar DM content of feed offered and feed refused.

7.3.2.2 Milk and body measurements: Milk yield (MY) and body weight (BW) were recorded daily automatically using the AfiMilk dairy farming system (AfiMilk Ltd, Kibbutz Afikim, Israel) at each milking throughout the trial. Body condition scores (BCS) were assigned weekly by 2 experienced persons using a 5-point scale (1 to 5 in 0.25-unit increments), as described by Wildman et al. (1982) and scores were averaged for each cow within 1 d of observation. Milk samples from individual cows were obtained weekly during PM milking (~15h00), preserved with broad spectrum microtabs and delivered to a SANAS accredited testing laboratory, MilkoLab (GE Dairy Supplies, Parow, Cape Town, South Africa) for milk components analysis. To take the AM milking (~04h00) into account, the Agricultural Research Council (ARC) formula for milk recording was used to convert the butterfat (De Waal, 2018). Based on milk sample analysis, the energy corrected milk yield (ECM) and fat corrected milk (FCM) were calculated as follows:

$$\text{ECM} = [0.327 \times \text{milk yield (kg/d)}] + [12.95 \times \text{fat yield (kg/d)}] + [7.2 \times \text{protein yield (kg/d)}]$$
 (Orth, 1992).

$$3.5\% \text{ FCM} = [0.4324 \times \text{milk yield (kg/d)}] + [16.216 \times \text{milk fat (kg/d)}]$$
 (Tyrrell and Reid, 1965).

7.3.2.3 Feeding behaviour monitoring: The rumination and eating time was measured using tri-axial accelerometer-based behaviour sensors supplied by Afimilk (AfiMilk Ltd, Kibbutz Afikim, Israel) fitted to neck collars. Rumination and eating data obtained with the automatic system were summarized daily and averaged at 5 day intervals of days in milk. Collars were attached to the neck of each cow at 21 days before calving. Only data collected from calving to 30 DIM were used for statistical analysis.

7.3.2.4 Blood samples: Blood samples were collected immediately after AM milking but prior to feeding via coccygeal venipuncture into BD Vacutainer K2E (EDTA) blood collection tubes (BD, Plymouth, PL6 78P, UK) which was kept on ice until centrifugation (within one hour from blood collection) at 3,000 rpm at 4 °C for 15 min to separate plasma. Blood glucose was measured immediately during blood sampling using the Bayer Contour™ TS (Bayer HealthCare (PTY) Ltd, South Africa) blood glucose meter in combination with Contour™ TS blood glucose test strips (Bayer HealthCare (PTY) Ltd, South Africa). Aliquots of plasma were stored at –20 °C until subsequent analysis for non-

esterified fatty acids (NEFA). Blood samples were collected weekly from 3 weeks prepartum and 3 weeks postpartum from each cow.

7.3.2.5 Fecal samples (approximately 250 g) were collected weekly from the rectum via rectal palpation and stored at -20°C until further processing and analysis. Samples were thawed at room temperature, pooled by pen and thoroughly mixed by hand. A subsample (~ 500 g) was weighed and dried at 60°C in a forced-air oven for 72 hours to determine DM. After drying, faecal samples were ground to pass through a 1-mm sieve using a Wiley mill (Model 4, Thomas-Scientific, USA) pending chemical analysis.

7.3.3 Chemical analysis

The DM and ash content were determined according to the AOAC (2002), official Method 934.01 (100°C ; 24h) and 942.05 (500°C ; 6h), respectively. The starch content was determined as described by Hall (2009). Crude protein (CP) was determined by the Dumas combustion method according to the AOAC, official method 992.15 using a LECO nitrogen analyser (model FP-528, Leco Corporation, St. Joseph, MI, USA) and ether extract (EE) by using a Tecator Soxtec System HT 1043 Extraction Unit (AOAC, 2002; Method 920.39). Neutral detergent fiber (NDF) was analysed as described by Mertens (2002) using sodium sulfite and thermostable α -amylase, and acid detergent lignin (ADL) was determined according to Goering and Van Soest (1970) as modified by Raffrenato and Van Amburgh (2011). Organic matter (OM) was calculated as $100 - \% \text{ash}$; Non-fiber carbohydrates (NFC) was calculated as $100 - (\% \text{CP} + \% \text{NDF} + \% \text{EE} + \% \text{ash})$ according to the NRC (2001). Potentially digestible NDF (pdNDF) was calculated as $\text{NDF} - \text{iNDF}$. The indigestible NDF (iNDF) in all TMR, Orts and fecal samples was determined using a long term (240 h) *in vitro* fermentation as described by Raffrenato et al. (2018). All samples were analysed in duplicate.

Milk samples were analysed for fat, protein, lactose, somatic cell count (SCC) and milk urea nitrogen (MUN) by means of infrared analysis (CombiFossTM FT+, Hillerød, Denmark) at MilkoLab (GE Dairy Supplies, Parow, Cape Town, South Africa). Non-esterified fatty acids (NEFA) were determined by means of an *in vitro*

enzymatic colorimetric method assay by using the Radox FA115 reagents (Radox Laboratories, Ltd, South Africa) and a Cobas Integra 400 plus analyzer (Roche Diagnostic, Ltd, South Africa).

7.3.4 Calculations and data analysis

Daily pen DMI was measured as the difference between the as fed feed offered and as is feed refused (orts) multiplied by the DM content of the TMR. The number of cows per pen per day was used to calculate mean individual intake. Body weight change was calculated as the difference between BW from day 1 to 30 DIM. Change in BCS before calving was calculated from as the difference between BCS at 21 d before calving to calving and change in BCS after calving was calculated from day 1 to 30 DIM. Before statistical analyses all the variables measured at different days in milk were condensed to 5-DIM interval means from -20 to +30 DIM (i.e. -20, -15, -10, -5, +5, +10, +15, +20, +25, +30).

Data were analysed using SAS software (version 9.3; SAS Institute Inc., Cary, NC, USA). Even if the animals were assigned to either the control or treatment diet after calving, pre-partum data was also analysed for graphical presentation before calving for all the response variables analysed, except for milk yield and composition. However, to avoid problems with fitting a covariance structure in the statistical model and because the diet treatment was applied only after calving, data pre and post-partum were analysed separately and values from the day of parturition (± 2 days) were not included. When analysing intake, pen was considered the experimental unit, while with the other data cow represented the experimental unit. Body weight, BCS, eating and rumination time, glucose, NEFA, milk yield and composition, SCC and MUN were analyzed with PROC GLIMMIX of SAS (version 9.3; SAS Institute Inc., Cary, NC, USA) as a completely randomized design with a factorial arrangement of treatments with covariate adjustment corresponding to the response variable in the pre-partum period or in the previous lactation when available. The model included the fixed effects of diet, parity, 5-DIM interval and the respective 2-way interactions. The 3-way interaction was removed from the model because of non-significance. The five-DIM interval was included in the model

as a repeated measure using the compound symmetry covariance structure which provided the best fit based on the Akaike's information criterion (Littell et al., 1998). Cow nested within parity and diet was added as random effect, except for DMI. No repeated measure effect was included for BW change and BCS change. Degrees of freedom were calculated using the Kenward-Roger option. Least squares means were determined and treatment means within 5-DIM intervals compared using the SLICE option. Statistical significance and trends were considered at $P \leq 0.05$ and $P > 0.05$ to $P \leq 0.10$, respectively.

7.4. Results and Discussion

7.4.1. Nutrient composition of the experimental diets

The ingredients and chemical composition of the four experimental diets are presented in Table 7.1. Although diets were formulated to contain similar starch concentration (28 %), the analysis of the TMR differed, with lower starch content in the control diet (3 mm ground maize; MF) (25.44%) as compared to the treatment diet (6 mm ground maize; MC) (27.72%). A lower starch content of TMR as compared to formulated diets could be due to differences in methods used in the determination of starch content of raw materials and in the TMR. Raw materials were analysed at a feed company to obtain nutrient content information for ration formulation. In the feed industry in South Africa most companies use the Near Infrared Reflectance Spectroscopy (NIR) instead of wet chemistry, which is used in our laboratory. The wet chemistry method is more accurate and precise compared to NIR which relies heavily on frequent precision calibrations with large calibration datasets of local feeds (Corson et al., 1999). The crude protein, NDF, iNDF, ADL, EE and ash content were similar across diets and averaged 17.31, 35.55, 12.63, 3.17, 2.65 and 7.72 % (DM basis), respectively. Overall, the chemical composition of the diets was similar, with the exception of the starch which was 2.28 % lower in the control diet.

7.4.2. Intake and feeding behaviour

The aim of this study was to evaluate the effects of starch fermentability by varying the maize particle of diets fed during the early postpartum period on DMI, performance and metabolites of fresh cows. The specific objective was to partially shift site of starch digestion. Typically, feed intake is decreased by ~30% during the early PP period (Bertics et al., 1992; Bell, 1995). According to the hepatic oxidation theory (HOT), highly fermentable starch sources decrease feed intake because of increased ruminal propionate, hence the supply of propionate to the liver, stimulating oxidation of fuel in the liver (Allen, 2000). Moreover, it has been supported that the hypophagic effects of propionate is intensified during the early PP period when cows are in a lipolytic state with elevated concentration of plasma non-esterified fatty acids (NEFA) and greater content of hepatic acetyl CoA (Oba and Allen, 2003b; Allen et al., 2009; Stocks and Allen, 2012, 2013; Piantoni et al., 2015). In this study, diets were formulated to vary only in particle size of maize to avoid confounding effects, such as from interactions between diet starch concentration and fermentability as well as differences in diet forage NDF content which have shown conflicting results reported in previous studies (Albornoz and Allen, 2018).

Dry matter intake (DMI), eating and rumination time are presented in Table 7.4. The DMI was affected by diet ($P = 0.0330$). Cows fed the MF diet had lower DMI compared with cows fed the MC diet (16.08 vs. 17.13 kg/d). Parity x diet interaction was not significant but, as expected, multiparous cows consumed more than the primiparous cows. Diet did not affect eating and rumination time across the whole period. On average, the eating time was 581.13 ± 7.48 and 583.57 ± 7.59 min/d and the rumination time was 308.58 ± 5.88 and 315.35 ± 5.97 for MF and MC, respectively. The eating and rumination patterns for cows fed finely and coarsely ground maize is presented in Figure 7.1. Although there were fluctuations in the eating time during the first 30 days in milk, cows fed the MC showed less fluctuation than cows fed the MF diet. Interestingly, diet significantly interacted with DIM for both eating ($P = 0.0008$) and rumination time ($P = 0.0019$), implying that cows change their feeding behavior as lactation progresses.

The lower DMI in cows fed MF diet compared with MC diet is in agreement with previous studies (Dann et al., 1999; Sadri et al., 2009; Albornoz and Allen, 2018), supporting the hepatic oxidation theory of the control of feed intake (Allen et al., 2009).

Albornoz and Allen (2018) reported that increasing diet starch fermentability by substituting high moisture corn (HMC) for dry ground corn (DGC) decreased DMI during the first 23 d PP. This reduction was greater with high starch diets (18.17 vs. 17.7 and 20.2 vs. 16.3 in HMC vs. DGC in a low (22 %) and high (28 %) starch diets, respectively). In the current study the treatment diet was also slightly higher in starch content (27% vs. 25%) and therefore the particle size played a major role in the reduction of DMI. In agreement, Dann et al. (1999) found that decreasing diet starch fermentability by substituting steam-rolled maize for cracked maize from 1 to 63 d PP tended to increase DMI ($P = 0.13$), with cows fed cracked maize consuming 1.2 kg more than the steam-rolled maize. Similarly, Sadri et al. (2009) reported that increasing diet starch fermentability by substituting dry ground barley with dry ground maize decreased DMI by 1.4 kg/d during the first 28 d PP. These differences were however not significant ($P = 0.53$). In contrast, Rockwell and Allen (2016) reported that increasing ruminal starch digestibility by substituting HMC for DGC did not affect DMI during the first 28 d PP. The lack of effects in the latter study could be possibly due to the high forage NDF content (27.4%) causing the DMI to plateau at 10 d PP instead of increasing steadily (Albornoz and Allen, 2018). Albornoz and Allen (2018) explained that forage NDF is very filling (Allen, 2000), and may have increased satiety by increasing ruminal distention rather than being controlled by metabolic effects associated with starch fermentability as observed by Rockwell and Allen (2016). Another possible explanation for lower DMI in the MF diet compared to the MC diet observed in our study is that the cows fed the MF diet might have been more prone to sub-clinical acidosis because of high starch fermentation, which will have an influence on DMI.

Most of the previous studies introduced other processing types, besides milling. South African feed companies instead do not diversify their grain maize which is usually milled with either a 3 or 4-mm sieve size, depending on the company. This was the main reason for focusing on particle size only. The resulting particle size distribution can however vary depending on the vitreousness and the mill used (Al-Rabadi et al., 2012; Gallo et al., 2016). Decreasing particle size by mechanical processing (grinding) increases fermentability of starch in the rumen (Ferraretto et al., 2013), thereby increasing production of propionate, which in turn suppresses feed intake. Finely ground maize was

more fermentable *in vitro* than the coarsely ground maize (Table 7.1). We expected the MF diet to increase production of propionate in the rumen and depress DMI compared with MC during the early PP period when cows were in a lipolytic state. This study also intended to reduce ruminal starch fermentability using coarsely ground maize, hence shifting some amounts of starch to be digested postruminally. Shifting the site of starch digestion postruminally is expected to increase feed intake. This is because fuels absorbed postruminally do not stimulate hepatic oxidation to the same degree as propionate and because of increased latency for fuels reaching the liver; transit time from the rumen to the intestines significantly delays fuel absorption (Albornoz and Allen, 2018). Gualdrón-Duarte and Allen (2018) and Gualdrón-Duarte and Allen (2017) demonstrated how fuels supplied by the ruminal fermentation and intestinal digestion of starch have different effects on DMI that are consistent with their ability to stimulate hepatic oxidation of acetyl co-enzyme A.

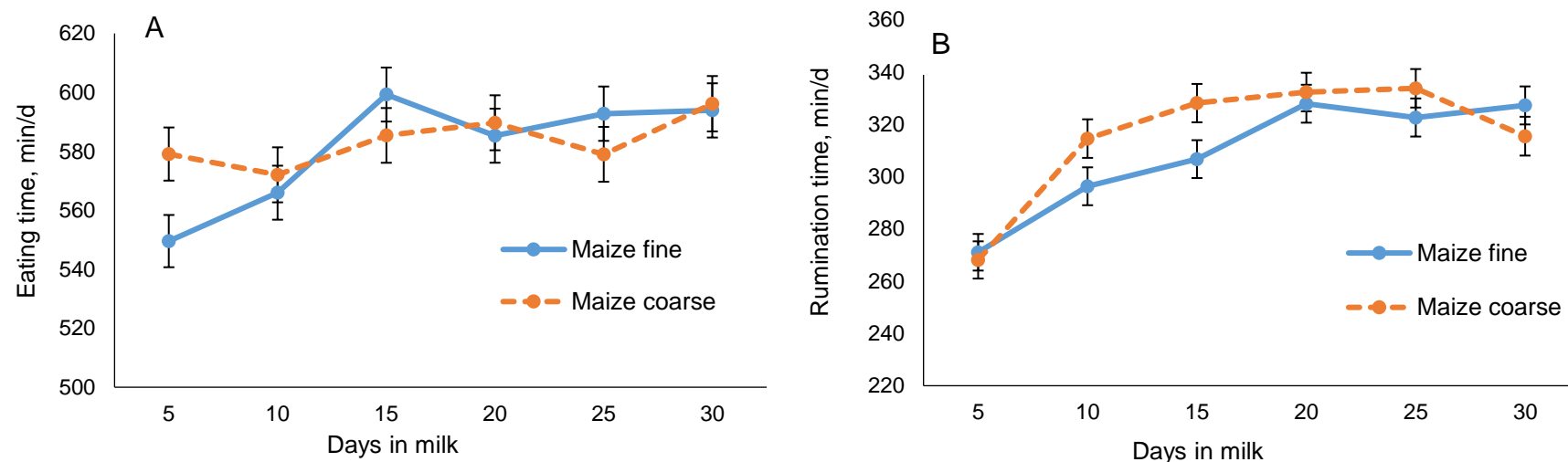
Propionate stimulate hepatic oxidation to the greatest extent because it is rapidly and efficiently extracted from the blood by the liver (Bell and Bauman, 1997; Reynolds et al., 2003). Propionate is further an obligate anaplerotic metabolite and have to always enter the tricarboxylic acid (TCA) cycle to be metabolized before contributing to gluconeogenesis (Gualdrón-Duarte and Allen, 2017, 2018). Therefore greater propionate flux to the liver would result in faster oxidation of acetyl coenzyme A, which can stimulate satiety and reduce feed intake (Maldini and Allen, 2018). In contrast, the primary fuel from digestion of starch in the small intestine (i.e. glucose) is unable to stimulate hepatic oxidation directly (Gualdrón-Duarte and Allen, 2018). Absorbed glucose is partially metabolized to lactate, with much lower liver uptake than propionate (Reynolds et al., 2003) and therefore have less effects on satiety than propionate (Albornoz and Allen, 2018; Gualdrón-Duarte and Allen, 2018).

Table 7. 3. Effects of starch fermentability by varying particle size of maize on DMI, rumination and eating behaviour of primiparous and multiparous cows during the first 30 days in milk.

Items	Finely ground maize		Coarsely ground maize		P-value					
	Primiparous	Multiparous	Primiparous	Multiparous	Parity	Diet	DIM	Parity x diet	DIM x Parity	Diet x DIM
DMI	13.64±0.49 ^d	18.51±0.49 ^b	14.54±0.49 ^c	19.72±0.49 ^a	<.0001	0.0330	N/A	0.7556	N/A	N/A
Eating time	617.07±11.33 ^a	545.20±9.76 ^b	612.12±11.62 ^a	555.01±9.76 ^b	<.0001	0.8198	<.0001	0.4901	0.2731	0.0008
Rumination time	297.76±8.91 ^b	319.40±7.68 ^{ab}	304.22±9.14 ^{ab}	326.47±7.67 ^a	0.0101	0.4213	<.0001	0.9711	0.1155	0.0019

Least squares means of rumination or eating times with different superscripts differ, $P < 0.5$.

MF= finely ground maize, MC = coarsely ground maize

**Figure 7. 1.** Changes in eating (A) and rumination (B) time during the first 30 days in milk for cows fed finely or coarsely ground maize.

7.4.3. Milk yield and composition

Milk production and composition are presented in Table 7.2. Milk yield was significantly affected by diet ($P = 0.0390$). The MC diet increased milk yield compared with the MF diet (21.70 vs. 20.41 kg/d). In previous studies with varying starch fermentability in early PP period, Alborno and Allen (2018) reported that feeding a less fermentable starch source (DGC) during the early PP period (first 23 DIM) increased milk yield compared with a highly fermentable starch source (HMC) (40.6 vs. 37.0 kg/d). However, Sadri et al. (2009) found that milk yield was not affected by substituting ground barley with ground maize. In agreement, Santos et al. (1999) reported that differences in rumen degradability of starch of grains (steam-flaked sorghum vs. steam-rolled maize) had no significant effects on milk yield in the first 45 DIM. Conversely, Rockwell and Allen (2016) reported that high moisture maize increased milk yield for the first 28 DIM compared to dry ground maize (41.4 vs. 38.5 kg/d). Dann et al. (1999) also observed that cows fed steam-flaked maize postpartum produced 2.3 kg/d more milk than cows fed cracked maize during the first 63 DIM. Typically, milk yield can be increased either by increasing ruminal starch digestibility, increasing the production of propionate and increased microbial protein synthesis, by increased postruminal digestion of starch, or increased bypass protein or by a combination of the mentioned factors (Dann et al., 1999). The increased milk yield observed in this study could be attributed to the shifting of site of starch digestion, hence increased postruminal digestion of starch. Increases in postruminally degraded starch has a larger effect on milk production compared to an increase in ruminally degraded starch (Nocek and Tamminga, 1991). Increased milk yield in this study could also be attributed to DMI. Cows fed the MC diet increased DMI. Furthermore, the MC diet provided a slightly higher amount of starch, due to both higher starch content and higher intake. Diets increased milk yield over time ($P < .0001$), with coarsely ground maize increasing milk yield more as compared with finely ground maize as time progressed (Diet x DIM; $P = 0.0008$) (Figure 7.2). The differences in milk yield were significant from day 15 to 25.

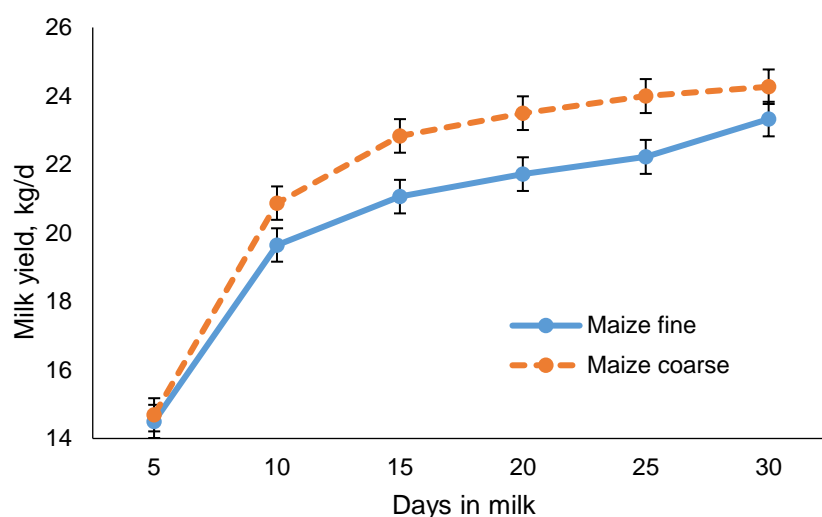


Figure 7. 2. Effects of starch fermentability by varying particle size of maize on milk yield during the first 30 days in milk.

The effects of diets on milk composition are presented in Table 7.4 and the effects of diets on milk fat, protein, 3.5% FCM and lactose over the first 30 DIM are shown in Figure 7.4. The MF tended to increase milk fat percentage ($P = 0.0575$) compared with MC (5.57 vs. 5.27 %) but had no effects on milk fat yield. On average, milk fat yield was 1.12 ± 0.04 and 1.13 ± 0.03 , kg/d in MF and MC, respectively. Multiparous cows fed the MF diet had the highest milk fat percentage (6.09 ± 0.19 %). There was no interaction observed between diets and parity nor between diets and DIM on milk fat percentage and yields. A DIM x parity interaction in milk fat percentage ($P = 0.0208$) indicated that multiparous cows had a higher milk fat concentration compared to primiparous cows at 5 DIM (8.39 vs. 4.67 %). Contrary to our results, Albornoz and Allen (2018) reported that increasing ruminal starch fermentability by substituting HMC for DGC decreased milk fat yield over time. Similarly, Dann et al. (1999) reported that steam-rolled maize decreased milk fat concentration compared with cracked maize. However, Rockwell and Allen (2016) and Sadri et al. (2009) reported that increasing diet starch digestibility had no effect on milk fat concentration or yield. Albornoz and Allen (2018) explained that the negative effects of HMC on milk fat was due to its suppression of DMI and milk yield rather than factors associated with milk fat depression. Ruminal starch digestibility is negatively correlated with milk fat concentration (Theurer et al., 1999; Firkins et al., 2001). Higher ruminal propionate produced from increased ruminal fermentability increases gluconeogenesis, which in turn, stimulates insulin secretion, thereby decreasing the amount of fatty acids that are released from adipose tissue to the mammary gland, thus decreasing milk fat (Bauman

and Griinari, 2003). Some studies reported that greater milk fat concentration is associated with greater loss in BCS (Janovick and Drackley, 2010). In this study, multiparous cows fed MF diets had higher milk fat concentration but lost less BCS. In this study, milk protein, MUN, ECM, 3.5% FCM and SCC was not affected by diets ($P>0.10$). This is in agreement to that of Rockwell and Allen (2016). Interactions were also not significant ($P>0.10$). On average, milk protein concentration and milk protein yield were 3.63 ± 0.05 and 3.61 ± 0.04 %, and 0.74 ± 0.02 and 0.76 ± 0.02 kg/d for MF and MC, respectively. Dann et al. (1999) also reported that milk protein concentration and yield for the first 63 DIM were not affected by diet. Rockwell and Allen (2016) found that milk protein yield was not affected by diet (1.27 vs. 1.32 kg/d), but HMC tended to decrease concentration of protein in milk compared to ground maize (3.00 vs. 3.11 %). Unlike milk fat concentration, milk protein concentration is highly correlated with ruminal starch digestibility (Theurer et al., 1999; Firkins et al., 2001). An increase in milk protein content, associated with an increase in ruminally starch digestibility reported in some studies has been attributed to increased microbial protein synthesis (Theurer et al., 1999). Apart from NEB, cows in the early PP period are typically in negative protein balance, which may impact milk protein synthesis (Dann et al., 1999).

Insufficient crude protein intake or certain amino acids may cause a lack of milk protein response during the early lactation period (DePeters and Cant, 1992). The average for other constituents was 9.61 ± 0.36 and 10.28 ± 0.29 mg/dL for MUN, 26.91 ± 0.73 and 27.67 ± 0.64 , kg/d for ECM, and 27.07 ± 0.80 and 27.75 ± 0.69 , kg/d for 3.5% FCM in MF and MC diets, respectively. Dann et al. (1999) found that the concentrations of MUN were lower for cows fed steam-flaked maize compared to a cracked maize based diets fed postpartum. The latter authors attributed their result to improved nitrogen utilization from steam-flaked maize. The percentage of lactose was lower in the MF compared to the MC diet (4.61 vs. 4.70; $P = 0.0138$) and lactose production was lower (0.94 vs. 1.01 kg/d), in agreement with what was observed by Rockwell and Allen (2016) and Dann et al. (1999). A diet \times parity interaction ($P = 0.0492$) for lactose percentage suggested that the lower production was more pronounced in multiparous cows fed the MF than in primiparous cows (4.55% lactose for multiparous cows fed MF vs. 4.70 % for multiparous cows fed MC). Overall, concentrations of ECM, 3.5% FCM and lactose increased over time, MUN and SCC were not affected by DIM, whereas milk fat and protein percentage was highest at 5 DIM compared to other days.

Table 7. 4. Effects of starch fermentability by varying particle size of maize on milk yield and composition during the first 30 days in milk.

Items	Finely ground maize		Coarsely ground maize		P-value								
	Primiparous	Multiparous	Primiparous	Multiparous	Parity	Diet	DIM	Diet	x	DIM	x	Diet	x
								Parity	Parity	DIM	DIM		
Milk yield, kg/d	16.42±0.06 ^d	24.41±0.05 ^b	17.89±0.07 ^c	25.50±0.05 ^a	<.0001	0.0390	<.0001	0.7530	<.0001			0.0008	
Fat, %	5.05±0.15 ^c	6.09±0.20 ^a	4.87±0.15 ^c	5.68±0.14 ^b	<.0001	0.0575	0.0722	0.3285		0.0208		0.2264	
Fat, kg/d	0.84±0.05 ^b	1.41±0.06 ^a	0.87±0.05 ^b	1.38±0.05 ^a	<.0001	0.9957	<.0001	0.4743		0.3668		0.5166	
Protein, %	3.57±0.05	3.69±0.06	3.54±0.05	3.68±0.05	0.0375	0.7402	<.0001	0.8828		0.4642		0.3276	
Protein, kg/d	0.59±0.02	0.88±0.02	0.61±0.02	0.91±0.02	<.0001	0.3559	<.0001	0.9353		0.0066		0.3795	
Lactose, %	4.66±0.03 ^a	4.55±0.04 ^b	4.69±0.03 ^a	4.70±0.03 ^a	0.1910	0.0138	<.0001	0.0492		0.0278		0.5855	
Lactose, kg/d	0.78±0.03 ^c	1.10±0.03 ^b	0.83±0.03 ^c	1.18±0.03 ^a	<.0001	0.0266	<.0001	0.5595		0.0012		0.3378	
MUN, mg/dL	9.55±0.42	9.67±0.55	10.65±0.41	9.90±0.41	0.5259	0.1312	0.4118	0.2336		0.0015		0.9988	
SCC, x 1000/ml	285.17±197.94	425.63±233.92	243.72±198.59	608.16±184.92	0.2590	0.7064	0.8954	0.5141		0.9624		0.5895	
3.5% FCM ¹	20.81±1.00	33.33±1.17	21.96±1.00	33.55±0.93	<.0001	0.4962	<.0001	0.6049		0.2919		0.5970	
ECM ²	20.83±0.94 ^b	32.99±1.06 ^a	21.96±0.94 ^b	33.37±0.86 ^a	<.0001	0.4160	<.0001	0.6729		0.1807		0.7642	

^{a-c}values within a row with different superscript differ (P < 0.05).

¹3.5% FCM == [0.4324 × milk yield (kg/d)] + [16.216 × milk fat (kg/d)].

¹ECM (energy-corrected milk) = [0.327 × milk yield (kg)] + [12.95 × fat yield (kg)] + [7.2 × protein yield (kg)] (Orth, 1992)

²Feed efficiency = ECM/DMI

MY = Milk yield; MUN = Milk urea nitrogen; DIM = Day in milk, as from calving

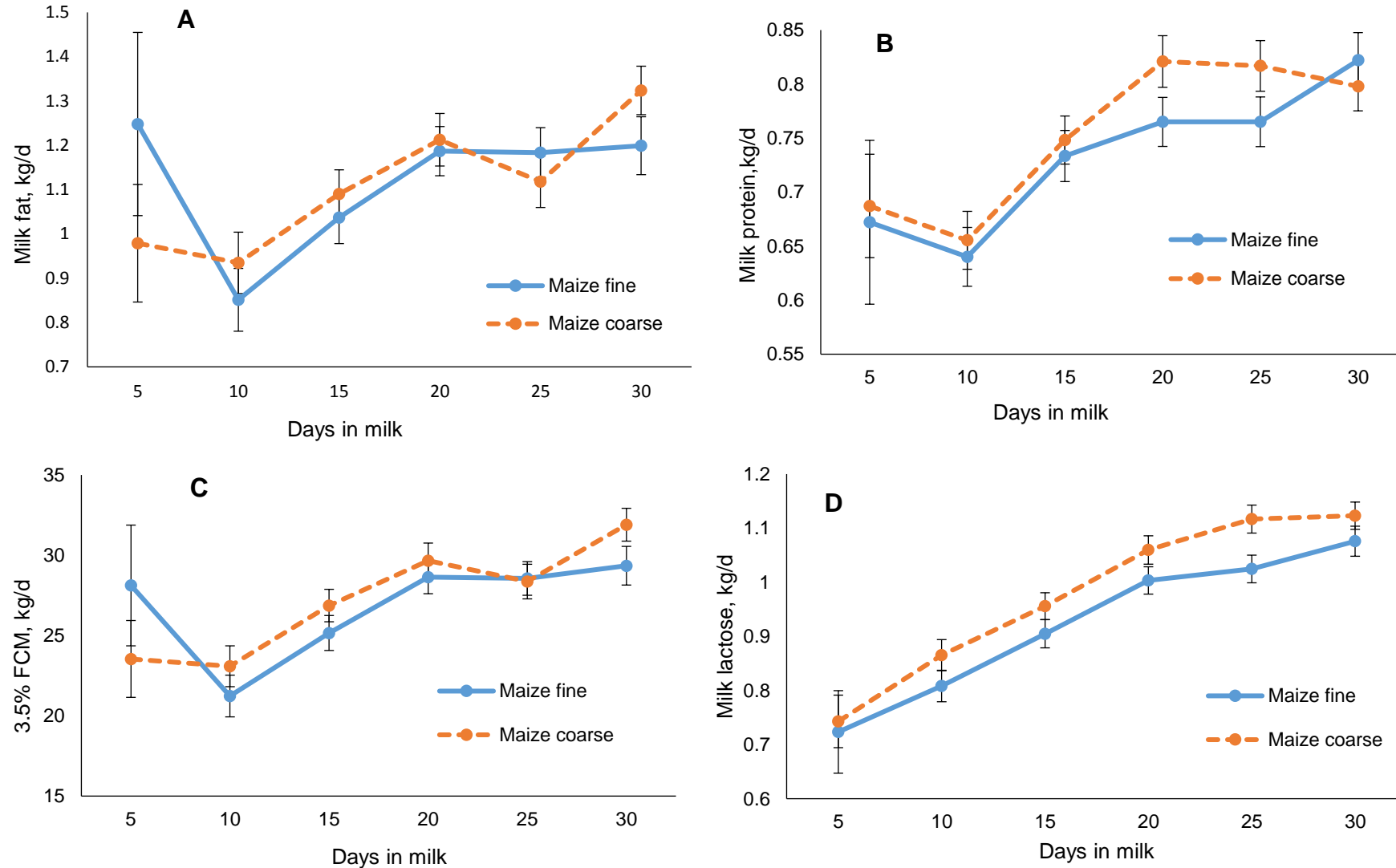


Figure 7. 3. Effects of starch fermentability by varying particle size of maize on milk fat yield (A), milk protein yield (B), 3.5% FCM yield (C) and milk lactose (D) during the first 30 days in milk.

7.4.3. Body measurements, and blood metabolites during the prepartum and early PP period

Body condition score, glucose concentration and non-esterified fatty acid (NEFA) of primiparous and multiparous cows during the prepartum period (21 d before calving) are presented in Table 7.5. On average, the BCS for the cows assigned to the MF and MC diet was 3.58 ± 0.05 and 3.59 ± 0.05 , glucose was 2.91 ± 0.10 and 2.89 ± 0.07 mmol/L and NEFA was 0.18 ± 0.02 and 0.15 ± 0.01 mmol/L, respectively. Primiparous and multiparous cows averaged 3.64 ± 0.05 and 3.51 ± 0.05 for BCS, 3.01 ± 0.07 and 2.79 ± 0.11 mmol/L for glucose, and 0.17 ± 0.01 and 0.17 ± 0.02 mmol/L for NEFA, respectively. There were no significant effects of parity on BCS, glucose and NEFA prepartum. However, BCS and glucose concentration tended to be greater in primiparous than multiparous cows ($P = 0.0712$ and $P = 0.1028$, respectively). A lower level of glucose in multiparous compared to primiparous cows indicates a higher demand of glucose by the mammary gland for the synthesis of colostrum. For primiparous cows, the mammary gland is less developed with fewer secretory gland as compared to multiparous cows (Kessler et al., 2014). The parity x DIM interaction tended ($P = 0.0955$) to affect glucose concentration, with primiparous cows having the highest glucose (3.28 mmol/L) at 20 days before calving. It is important to note that cows were fed the same close-up diet and were maintained under the same management conditions.

Table 7. 5. Body condition score, glucose and non-esterified fatty acids for primiparous and multiparous cows during the prepartum period (21 days) as per assigned postpartum diet.

Items	Finely ground maize		Coarsely ground maize		P-value			
	Primiparous	Multiparous	Primiparous	Multiparous	Parity	Diet	DIM	DIM x Parity
BCS	3.66 ± 0.07	3.47 ± 0.08	3.63 ± 0.07	3.55 ± 0.06	0.0712	0.7646	0.8860	0.6962
Glucose, mmol/L	3.09 ± 0.10	2.73 ± 0.17	2.93 ± 0.09	2.8511 ± 0.11	0.1028	0.8645	0.8994	0.0955
NEFA, mmol/L	0.18 ± 0.02	0.19 ± 0.03	0.15 ± 0.02	0.14 ± 0.02	0.9261	0.1281	0.1695	0.3841

The effects of starch fermentability by varying maize particle size on BW, BW change, BCS, BCS change, glucose and NEFA for primiparous and multiparous cows are presented in Table 7.6. All BW, BCS and NEFA were affected by diet, while the

concentration of glucose was similar between diets ($P = 0.6778$). The MF diet resulted in a lower BW and BCS compared with the MC diet (396.00 vs. 405.74 kg and 3.42 vs. 3.50, respectively). Similarly, changes in BW ($P = 0.0001$) and BCS ($P = 0.0026$) were affected by diet. Cows fed the MF diet lost more BW and BCS compared to the MC diet cows (39.92 vs 32.24 kg and 0.23 vs. 0.14 units), possibly because of lower DMI. Albornoz and Allen (2018) found that HMC decreased BW compared with DGC, but had no effects on BCS and changes in BCS and BW. Rockwell and Allen (2016) reported that HMC tended to increase BW compared with ground maize during the first 28 DIM, but not BCS, which was not affected by treatment. Other studies reported no significant changes in BW, BW and BCS change (Dann et al., 1999; Sadri et al., 2009). The magnitude of change in BW and BCS differ among diets in primiparous and multiparous cows (parity \times diet; $P = 0.0465$ and $P = 0.0213$, respectively). Primiparous cows fed the MF diet lost more BW and BCS compared with those fed the MC diet (55.43 vs. 46 kg and 0.26 vs. 0.20 units). The multiparous cows fed the MF diet lost more BW (24.41 vs. 18.48 kg) compared with the multiparous cows fed the MC diet, but they lost less BCS (0.11 vs. 0.18). Typically, dairy cows lose BCS during the early PP period because feed intake is inadequate to support the rapid increase in energy required for milk production, hence a negative energy balance (Leblanc, 2010). According to Broster and Broster (1998), the magnitude of loss in BCS postpartum is influenced by several factors, such as the BCS at calving and milk yield and milk fat yield. Cows with a greater BCS at calving lose more BCS postpartum and cows with higher milk yield often have greater loss in BCS (Roche et al., 2009). A greater loss in BCS postpartum is associated with greater mobilization of body reserves. The changes in BCS during the prepartum and during the first 30 days in milk is presented in Figure 7.4.

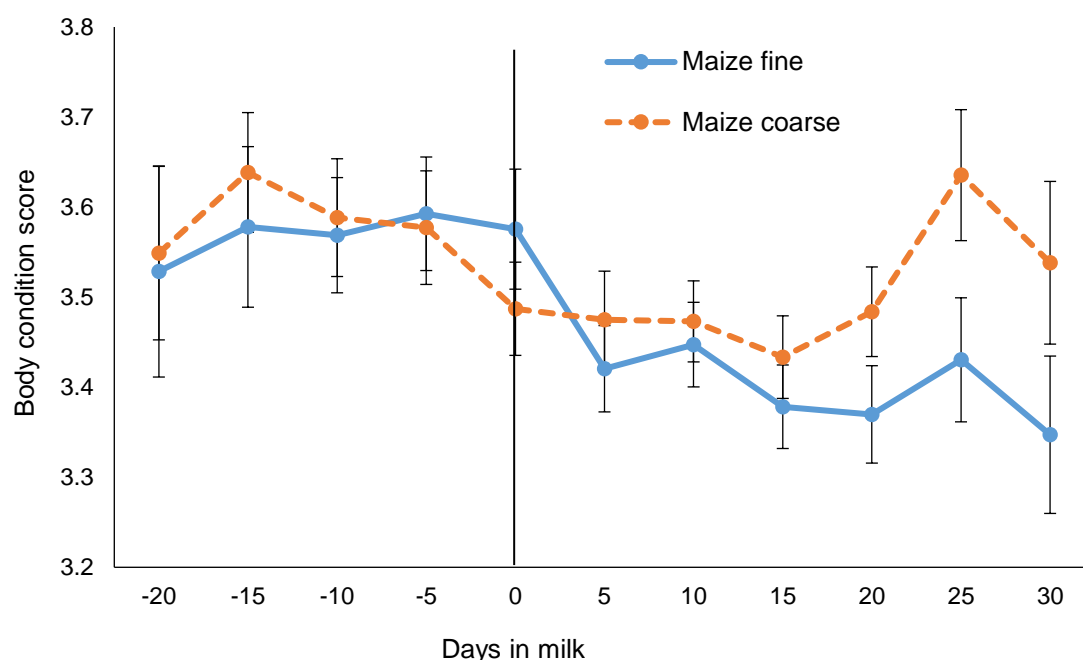


Figure 7. 4. Body condition score of dairy cows prepartum and effects of starch fermentability by varying particle size of maize on postpartum body condition score. The line at day 0 indicates the calving date, when cows received the experimental diets.

Although the level of glucose was not affected by diets, the concentration of glucose changed over time, with higher values around calving for both diets followed by a drop in the concentration post-partum and a subsequent rise at 30 DIM in cows fed the MF diet (Figure 7.5). There was no interaction between diet and DIM nor between diet and parity for glucose concentration. Previous studies have reported that glucose concentrations do not usually vary greatly because of nutritional modifications of the diet (Dann et al., 1999). The decreased glucose concentrations postpartum were to be expected. In fact, as explained by Ingvarsen (2006), although the intake of glucose into the adipose and muscular tissue is decreased while gluconeogenesis increases, as well as the use of non-esterified fatty acids and ketone bodies is increased to meet the demand of lactose production, the concentration of glucose normally lowers postpartum, especially in multiparous cows.

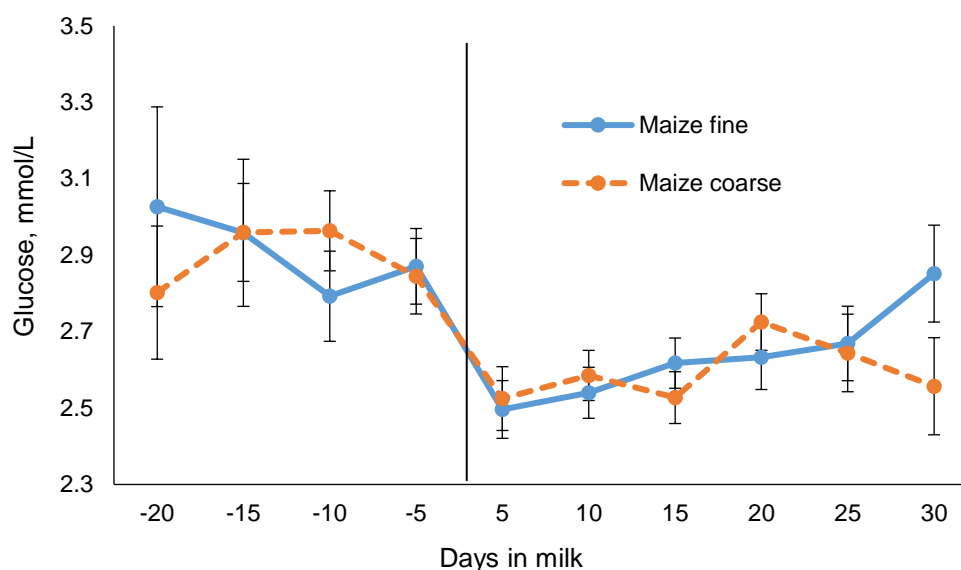


Figure 7. 5. Blood glucose concentration of dairy cows prepartum and effects of starch fermentability by varying particle size of maize on postpartum blood glucose concentration. The line indicates the calving date, when cows received the experimental diets.

Overall, the concentration of NEFA was low and stable before calving and high after calving but decreased as lactation progressed. In particular, although the concentration of plasma NEFA didn't reach ketotic levels, it was greater in cows fed the MF diet as compared to cows fed the MC diet (0.71 vs. 0.56 mmol/L; $P = 0.0176$). This result suggests that cows fed the MC diet were in a better metabolic status and in a lower negative energy balance. Possibly, cows fed less ruminal fermentable starch had a better utilization of dietary energy and therefore a reduction in body fat mobilization. Contrary to Lykos et al. (1997) who indicated that dietary energy from more rapidly fermentable starch had a better utilization, reducing mobilization of body fat. The concentration of NEFA increased from 5 days before calving (Figure 7.6) for both diets. In agreement with the literature, the concentration of NEFA increases rapidly as calving approaches and it remains elevated during the first few weeks of lactation (Le blanc, 2010). This is attributed to mobilization of body reserves before or immediately after calving because of high energy demand for foetal growth or milk production. Multiparous cows fed the MF diet increased plasma NEFA concentration more compared to the rest of the cows (Table 7.6), which could be due to lower DMI coupled with higher milk yield. There was a diet x DIM interaction ($P = 0.0093$), with cows fed the MF diet increasing plasma NEFA concentration dramatically at 10 DIM (1.01 mmol/L) which then decreased at 20 DIM (0.43 mmol/L).

In previous studies, Albornoz and Allen (2018) found that glucose and NEFA were not affected by diet. Dann et al. (1999) reported that glucose and NEFA were similar between cracked maize and steam-flaked maize during the first 7 days postpartum, but the concentration of NEFA tended to be higher (15.8%; $P = 0.09$) for cracked maize for week 2 to 9 postpartum compared to steam-flaked maize. Other studies on the effects of starch fermentability during early PP period did not report the glucose and NEFA concentration (Sadri et al., 2009; Rockwell and Allen, 2016).

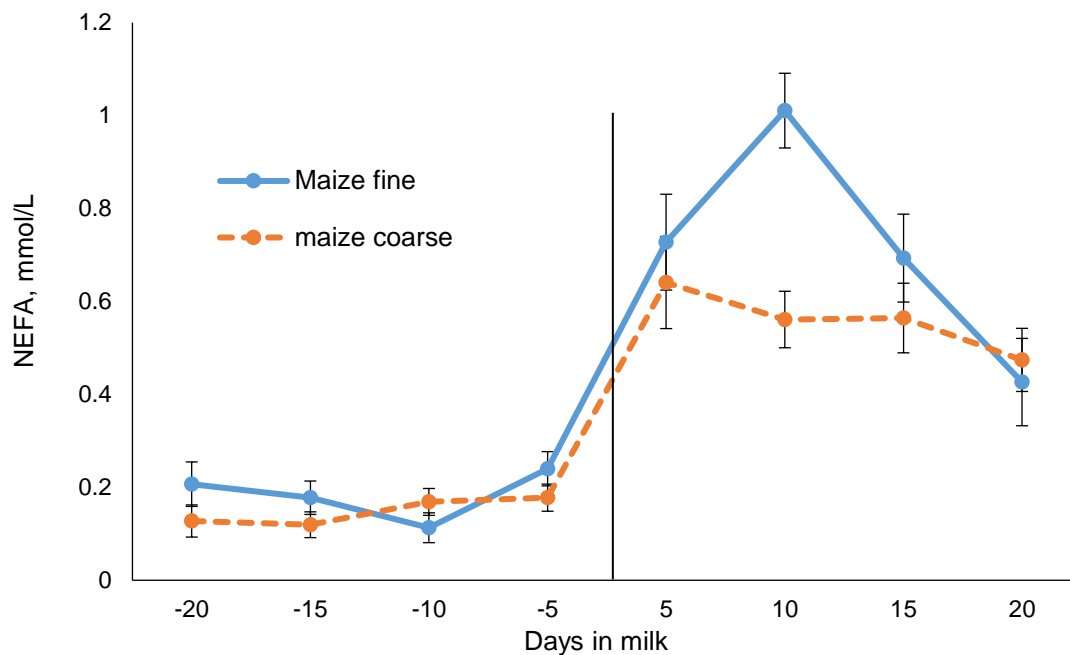


Figure 7. 6. Serum non-esterified fatty acid (NEFA) concentrations of dairy cows prepartum and effects of starch fermentability by varying particle size of maize on postpartum NEFA. The line between -5 and 5 DIM indicates the calving date, when cows received the experimental diets.

Table 7. 6. Effects of starch fermentability by varying particle size of maize on BW, BCS, glucose and non-esterified fatty acid for primiparous and multiparous cows during the first 30 days in milk.

Items	Finely ground maize		Coarsely ground maize		P-value					
	Primiparous	Multiparous	Primiparous	Multiparous	Parity	Diet	DIM	Diet x Parity	DIM x Parity	Diet x DIM
BW, kg	386.46±4.31 ^c	405.54±3.47 ^{ab}	397.16±4.61 ^{bc}	414.33±3.57 ^a	0.0001	0.0142	<.0001	0.8038	<.0001	0.1333
BW change, kg	55.43±0.96 ^a	24.41±0.67 ^c	46.00±1.00 ^b	18.48±0.71 ^d	<.0001	<.0001	N/A	0.0465	N/A	N/A
BCS	3.42±0.04 ^b	3.43±0.03 ^b	3.54±0.04 ^a	3.46±0.04 ^{ab}	0.3854	0.0461	0.1494	0.2437	0.8638	0.2536
BCS change	0.26±0.03 ^a	0.11±0.03 ^c	0.20±0.03 ^{ab}	0.18±0.03 ^{bc}	0.9841	0.0026	N/A	0.0213	N/A	N/A
Glucose, mmol/L	2.69±0.06	2.60±0.05	2.68±0.06	2.57±0.05	0.0675	0.6778	0.0364	0.8264	0.8732	0.5059
NEFA, mmol/L	0.58±0.07 ^b	0.85±0.07 ^a	0.52±0.06 ^b	0.60±0.06 ^b	0.0077	0.0176	0.0005	0.1415	0.0713	0.0093

7.5 Conclusion

Shifting the site of starch digestion in the early postpartum period may contribute to overcome problems encountered during this period. Reducing ruminal fermentability of starch by feeding coarsely ground maize increased dry matter intake and milk yield during the early PP period. This study supports the hepatic oxidation theory of the control of feed intake, which states that highly fermentable starch decreases feed intake. Diets had no effects on milk protein, ECM, 3.5 % FCM and MUN. Milk lactose was increased in the MC compared to the MF diet. Milk fat percentage tended to be greater in the MF compared to the MC diet, but it did not affect milk fat yield. The BW, BCS and NEFA were affected by diet, but the concentration of glucose was similar. Changes in BW and BCS were greater in cows fed finely ground maize, which in turn increased the concentration of plasma NEFA because of mobilization of body fat. Feeding coarsely ground maize decreased the concentration of plasma NEFA, suggesting that cows were in a better metabolic status, by reducing mobilization of body reserves. We observed some significant interactions in milk production and milk components, BW, BCS, glucose and NEFA. The increased DMI in the cows fed coarsely ground maize could possibly be attributed to reduced production of propionate in the rumen, shifting of digestion postruminally and by the decreased concentration of NEFA in the blood. Diets of dairy cows in the early postpartum period should be formulated to partially shift starch digestion postruminally by reducing the amount of starch digested in the rumen while maintaining a high total tract digestibility. This strategy may overcome problems encountered during this period by increasing feed intake, improving animal health, increasing milk yield and farm profitability.

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CHAPTER 8

Overall Conclusion and Recommendations

Dairy cows are fed a substantial amount of starch, supplied mainly by cereal grains such as maize, sorghum, wheat and barley to support milk production. Starch sources vary significantly in ruminal fermentability. According to the hepatic oxidation theory (HOT), highly fermentable starch sources increase the production of propionate which can suppress feed intake, especially during the early postpartum period when cows are in a lipolytic state. Shifting the site of starch digestion may overcome problems encountered by transition cows such as decreased feed intake, negative energy balance, impaired performance and metabolic and infectious diseases.

The main aim of the present dissertation was to investigate the potential practice and the effects of modulating site of starch digestion by varying ruminal fermentability of various starch sources on feed intake, and productive and metabolic response of transition cows. Different cereal grains and cultivars result in different particle size distributions and geometric mean particle sizes. Fractions of particle sizes of cereal grains obtained by sieving milled grains have the potential to provide the animal with different amounts of nutrients based on composition and starch digestion and possibly resulting in different energy and microbial protein. Starch degradability and rate of starch degradation increased with decreasing particle size of starch sources, suggesting that starch digestion could be shifted post-rumen based on particle size and thus reducing the amount fermented in the rumen, assuming that most starch will be digested post-rumen.

BioProtect™, a starch binding agent, was effective in decreasing ruminal starch degradability, with effects more pronounced on smaller particle size. Although BioProtect™ can reduce rumen starch degradation, the simulations (NDS software; CNCPS version 6.5, RUM&N, Reggio Emilia, Italy) of this study showed that larger particles were more effective at shifting the site of digestion and it could, therefore, be a more cost-effective option, assuming a high and consistent total tract starch digestibility.

The site of starch digestion can be shifted postruminally, but total tract starch digestibility could be decreased if ruminal digestion is not compensated for postruminally. Shifting the site of starch digestion postruminally by feeding coarse particle sizes (maize

milled using a 6 mm sieve) increased DMI, milk production and decreased mobilization of body reserves, indicated by decreased concentration of plasma NEFA in early postpartum cows. This supports the hepatic oxidation theory of control of feed intake, which states that highly fermentable starch decreases feed intake especially during the early PP period when cows are in a lipolytic state.

The recommendations of the present study are: 1) to better formulate diets for precision feeding by incorporating particle size as a factor in feed formulation programs to better characterize starch, 2) cereal grains fed to high producing ruminants should be sieved and fractions analysed for both composition and rumen degradability to improve their characterization, 3) cereal grains that are fed milled should be examined for distribution of particle sizes, chemical composition and digestibility whenever a different batch, grain or when a different feed mill is used because all add variability to the ration. Moreover, 4) diets of dairy cows especially during the transition period should be formulated to partially shift starch digestion postruminally by reducing the amount of starch digested in the rumen while maintaining a high total tract digestibility. This strategy could overcome problems encountered during the early postpartum period such as decreased feed intake and metabolic disorders, thereby increasing feed intake and milk yield and farm profitability.